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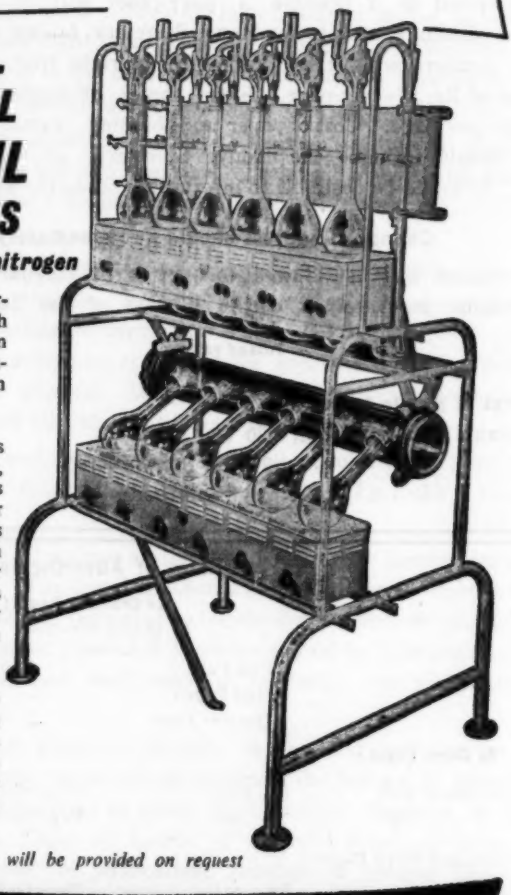

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[Jour. Indian Chem. Soc., Vol., 33, No. 4, 1956]

# THE CONSTITUTION OF CHAKSINE. PART I

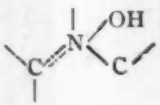
By S. K. Guha and J. N. Ray

On the basis of I. R. spectra of chaksine and its derivative, a structure for chaksine has been suggested. It appears that chaksine contains a bicyclic ring with a common N atom.

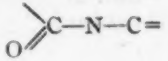
Siddiqui and Ahmed (*Proc. Ind. Acad. Sci.*, 1935, **2A**, 421) assigned the formula $C_{12}H_{21}O_2N_3$ for the alkaloid of *Cassia absus* isolated by them. But Ray *et al.* (this *Journal*, 1940, **17**, 281) suggested that the quaternary base should be formulated as $C_{11}H_{20}O_2N_3OH$ and adduced evidence in support of this view. The subsequent work of Siddiqui *et al.* (*J. Sci. Ind. Res.*, 1946, **4**, 101; vide also *Res. Bull. E. Panjab Univ.*, No. 21, p. 95) tacitly accepted the formula of Ray *et al.*

The present investigation deals with the determination of structure of chaksine. Chaksine has a quatrenary nitrogen, the two other nitrogen atoms are non-basic or at the most one is very feebly basic. Siddiqui *et al.* (*loc. cit.*) found that in alcoholic solution a disulphate of chaksine could be isolated, which was extremely susceptible to hydrolysis. This observation has been confirmed by us. Although sodium hydroxide solution liberates ammonia from chaksine very slowly even at 100°; baryta solution is more effective, and ammonia is copiously evolved on heating. The barium salts of acids obtained by baryta hydrolysis, when distilled with baryta, furnish a liquid showing a very strong pyrrole reaction. The oxidation with permanganate produces oxalic acid, an amino-acid (isolated as copper salt) and a lactone-like compound. Benzoylation of chaksine affords a dibenzoyl benzoate derivative but acetylation causes a breakdown of the molecule, a tri-acetyl derivative $C_9H_{11}O_2N_2.(CO.CH_3)_3$ being formed.

I.R. spectra of chaksine (Fig. 1) reveal the presence of C:N (6.35$\mu$) in the ring. Therefore, in chaksine



is postulated. The broad absorption in the region 3.0$\mu$ is due to bonded NH. This NH is part of an ureido group (absorption at 5.8$\mu$ and 5.95$\mu$). On heating chaksine sulphate with dilute hydrochloric acid, $CO_2$ is slowly liberated. This also suggests a grouping NH-CO-NH. The absorption at 7.85$\mu$ suggests the

grouping 

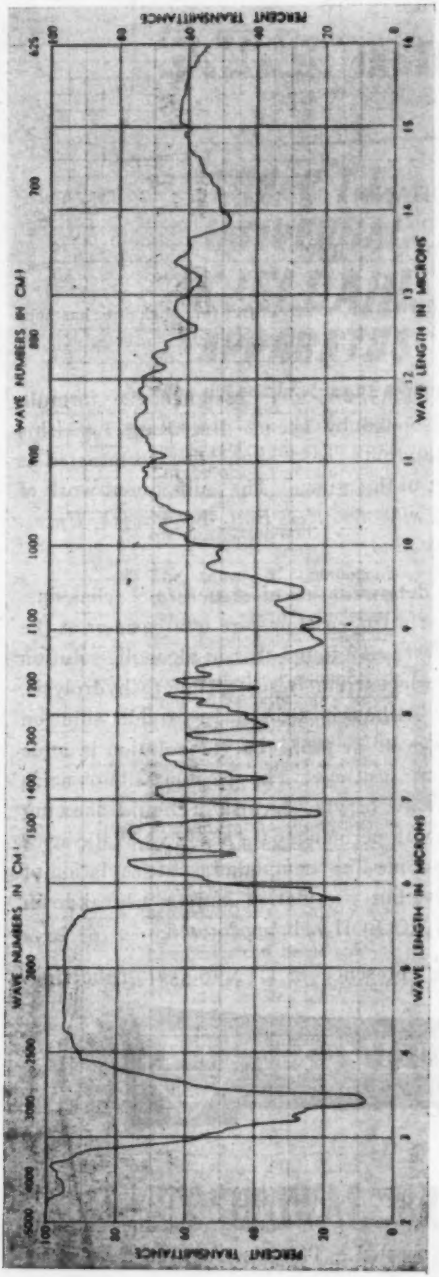



FIG. 1
(*Chaksine sulphate*).

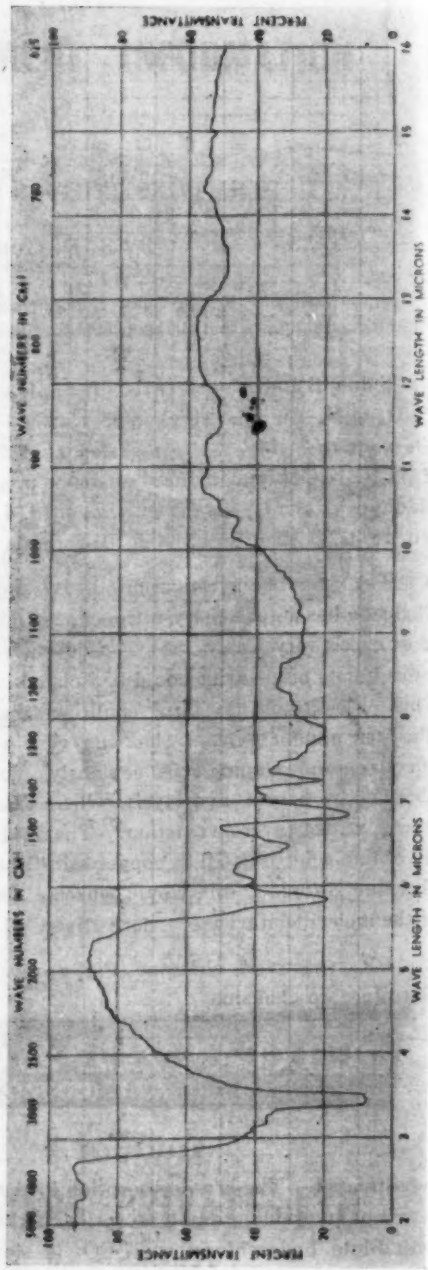



FIG. 2
(*Acid sulphate*).

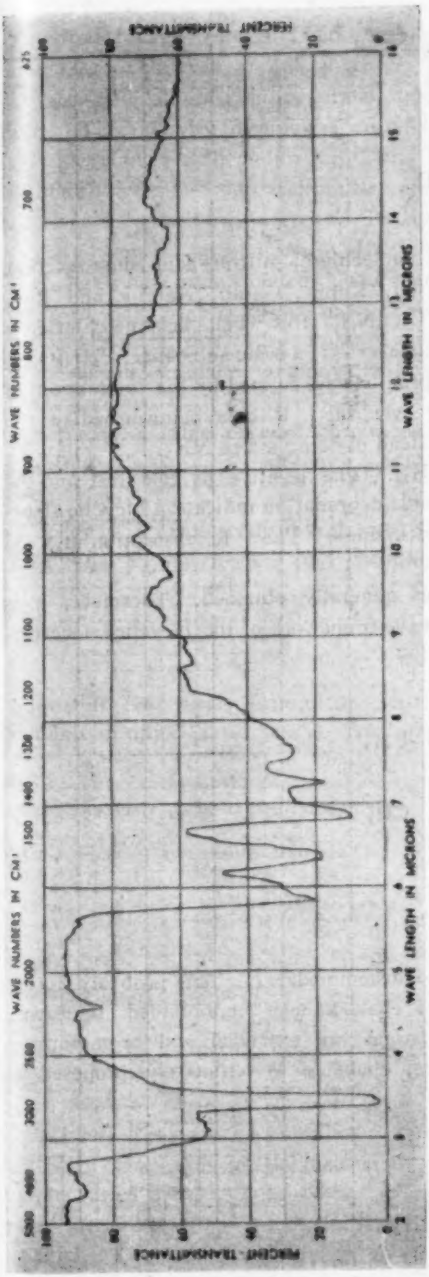



FIG. 3
(*Acid*)

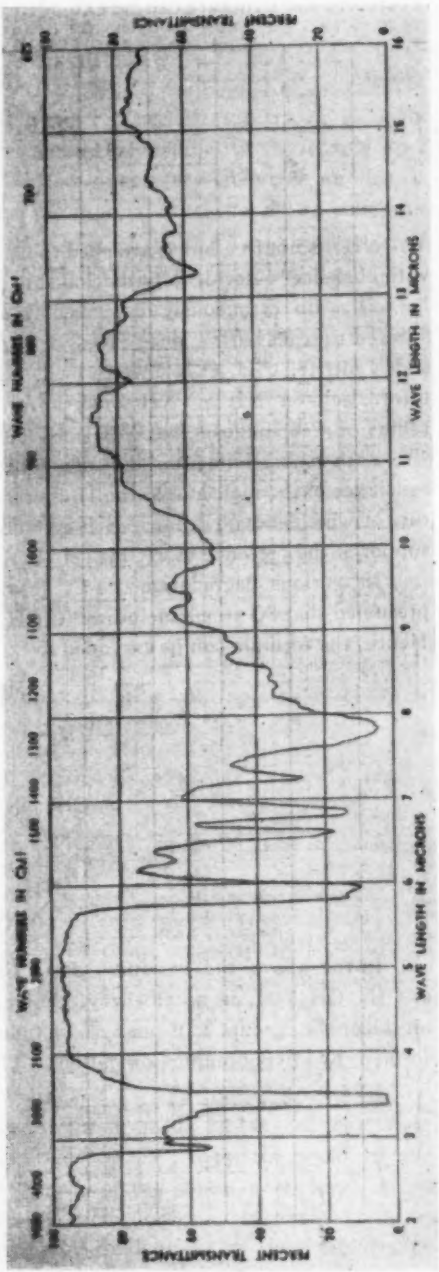
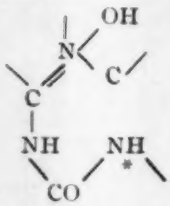



FIG. 4
(*Base*)

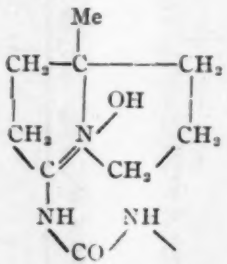
Therefore the structure can be extended to



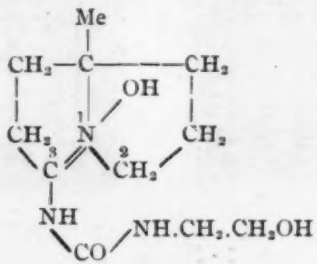
The NH* is not free but is attached to C, as one molecule of nitrous acid when reacting with chaksine chloride affords chaksine nitrite (cf. Kapur, Gaind, Narang and Ray, *loc. cit.*, who erroneously described it as $C_9H_{17}O_4N_3$). But when chaksine chloride is allowed to react with a large excess of nitrous acid at 0°-5°, a basic substance, $C_9H_{16}O_3N_2$ and a nitrite, $C_9H_{15}O_4N_3$, are formed. These products are formed by the interaction of nitrous acid on NH-CO-NH (*vide supra*). Moreover, the I. R. band being broad in the region of 3.0$\mu$ suggests that NH is associated instead of being a free $NH_2$.

Roth-Kuhn oxidation indicates one C.($CH_3$). The positive pyrrole test and the resistance of the molecule to the Hoffmann or Emde degradation indicate a bicyclic structure of which the quaternary N is common to both rings. The I. R. absorption at 7.22$\mu$ supports the presence of C-$CH_2$.

In various degradations, a $C_8$ residue was generally obtained. Therefore, it is presumed that 8C atoms including (C.$CH_3$) form the framework of the bicyclic structure. Hence, the formula can be extended to



In the above the residue $C_2H_5O$ has to be accommodated. This probably occurs as $CH_2$. $CH_2$.OH, as a tribenzoyl derivative of chaksine can be obtained indicating an alcoholic group. In many reactions 2 C atoms are extruded and bromoform is formed by hypobromite oxidation. Therefore, chaksine is tentatively proposed as

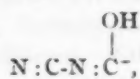


The double bond between 1 and 3 may be in equilbrium with its tautomer between (1) and (2).

Chaksine chloride shows curariform activity (De *et al.*, *J. Sci. Ind. Res.*, 1953, **12B**, 358). This activity diminishes and falls with time. The I.R. spectra of chaksine chloride show a band indicating C-Cl. This means the transition of Cl from N to C through the double bond making the molecule lose its quaternary character, and hence, also loss of curariform activity. By the action of fuming nitric acid on chaksine sulphate, several substances, $C_9H_{15}O_5N_3$, $C_6H_{10}O_3N_2$, $C_9H_{15}N_3O_2$, $C_9H_{15}O_3N_3$ and $C_9H_{17}N_3O_2$ have been isolated. These together with their structures, based on the formula proposed, have been described in the Experimental.

Chaksine nitrate with sulphuric acid furnishes several products, one of which is nitrochaksine sulphate (analogy with urea nitrate --➤ nitro-urea). There are other products, e.g. $C_{11}H_{16}O_5N_4$, $C_8H_{13}O_3N_3$. The latter has been oxidised to an acid, $C_8H_{10}O_4N_2$. Oxidation with chromic acid affords $(C_9H_{15}O_4N_3)_2SO_4$, but this substance probably is a mixture although it is crystalline and looks homogeneous under the microscope.

The U.V. spectrum reveals no distinctive features except that the inflection at 271m$\mu$ may be due to some kind of conjugation. This may arise from



a possible tautomeric form of the structure proposed. The I.R. spectra of chaksine sulphate in nujol "mull" suggest the presence of the following :

| Bands observed in chaksine sulphate. | Probable functional groups and structure assigned. | Bands observed in chaksine sulphate. | Probable functional groups and structure assigned. |
|---|---|---|---|
| 3.2 $\mu$ broad | Bonded NH | 7.62$\mu$ } | |
| 3.4 $\mu$ | Mull CH | 7.85$\mu$ } | |
| 5.8 $\mu$ | C :O conjugated ester | 8.0 $\mu$ } week | May be due to strained |
| 5.95$\mu$ } | -CO-NH- | 8.2 $\mu$ } | $\beta$-lactam. |
| 6.10$\mu$ } | | 8.3 $\mu$ } week | -CH-NH-CH |
| 6.35$\mu$ | C :N (ring) | 8.45$\mu$ } | and -CONH$_2$ |
| 6.80$\mu$ | Mull C-H | 8.80$\mu$ } | |
| 7.22$\mu$ | Mull C-H and C-CH$^3$ | 9.35$\mu$ } | $SO_4^{2-}$ |

## EXPERIMENTAL

*Isolation of Chaksine.*—In the present investigation seeds (about 500 lbs.) were extracted essentially by the method of Siddiqui *et al.* (*loc. cit.*, 1935) the yield of the iodide varied from 0.7% to 0.8%. The seeds from Bombay State give lesser yield than the seeds from North India. From the mother-liquors, *no iso*chaksine could be isolated. The pasty mass obtained by the method of Siddiqui *et al.* invariably gave chaksine oxalate with oxalic acid, yield 0.04%.

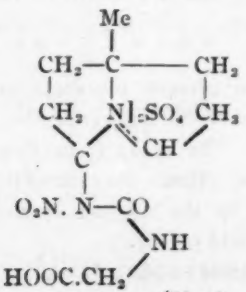
*Hydrolysis with Baryta.*—Chaksine sulphate (5.0 g.), suspended in water (100 c.c.), was treated with crystalline barium hydroxide (30 g. in water, 100 c. c.). The mixture

was stirred thoroughly at 60°. After filtration of separated barium sulphate, the solution was heated till the evolution of ammonia had ceased. The concentrated viscous solution was mixed with excess of baryta and dried in *vacuo*. The solids were only distilled affording an oily substance accompanied by much fumes. The ethereal solution of the distillate gave a strong pine shaving reaction, but quickly polymerised when evaporated. Hydrogen chloride converted the ethereal solution of the oil into an amorphous reddish substance, reminiscent of pyrrole red. No crystalline derivative could be prepared of the oil.

*Hydrolysis with dilute Hydrochloric Acid.*—Chaksine sulphate (1.0 g.) was refluxed with HCl (5%, 25 c. c.) for 3 hours. $CO_2$ was evolved. The neutralised solution afforded a minute amount of an oxalate, m.p. 132°, recrystallisable from hot water, but in quantities insufficient for characterisation.

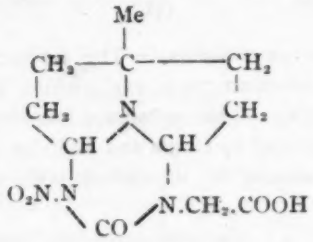
*Treatment of Chaskine Nitrate with concentrated Sulphuric Acid.*—To sulphuric acid (5 c. c . *d* 1.84) was added chaksine nitrate (2.5 g.) at 0° in small portions with stirring during ½ hour. The stirring was continued for 45 minutes, when a clear solution resulted. The mixture was poured into ice-cold water (150 c. c.). A white solid (I) (1.2 g.) separated and it was collected. The filtrate and washings were neutralised with sodium carbonate when a flocculent precipitate separated. Excess of sodium carbonate was avoided as a part of the precipitate redissolved in excess of the alkali. Filtration was avoided and the weak base was extracted with chloroform. The residue from chloroform was extracted with *N*-HCl. Reprecipitated from the acid extract with sodium carbonate, the base was collected (II, 0.8 g.).

Compound (I) was dissolved in a solution of sodium carbonate (6 g. in 40 c.c. water) by heating for ½ hour at 50°-70°. After standing for 3 hours, the filtered solution was reprecipitated with HCl avoiding excess of (III). The substance (I) was crystallised several times from hot dilute alcohol, m.p. 174° (decomp.). [Found : C, 39.3 ; H, 6.0 ; N, 17.0 ; S, 4.8. $(C_{11}H_{17}O_5N_4)_2\,SO_4$ requires C, 39.6 ; H, 5.2 ; N, 16.8 ; S, 4.8 per cent]. The Infra-red spectrum was studied in the form of "mull" in Nujol (Fig. 2). The broad absorption in the region 3.0$\mu$ can be due to strongly bonded H of NH or OH but it looks like more of a OH of a COOH group. Absorption at 3.4$\mu$ is that of mull. Absorption at 5.8$\mu$ and 6.1$\mu$ suggests the presence of a CO-NH group. Absorption at 6.5$\mu$ is due to a $NO_2$ group. Absorption at 7.23$\mu$ is due to C-$H_3$. The substance, (I) should be represented as follows on the basis of the structure proposed for chaksine.




(I)

The substance (III) was purified by slow crystallisation from acetone. decomposes in contact with hot solvents. It can be easily dissolved in sodium carbonate and reprecipitated without decomposition, m.p. 160° (decomp.) after crystallisation from acetone. (Found: C, 46 1 ; H, 6.3 ; N, 19.6 ; M. W. from Ba salt, 296. Calc. for $C_{11}H_{16}O_8N_4$: C, 46.5 ; H, 5.6 ; N, 19.7 per cent. M.W., 284). The I.R. spectra (Fig. 3) is practically the same as that of substance (I), except that the $SO_4^{2-}$ band is absent and there is a band at 4.6$\mu$. The latter is due to C≡N. Therefore, (III) is

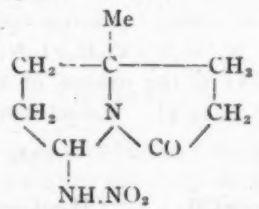


(III)

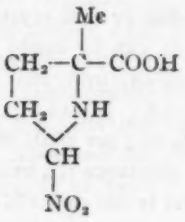
The base (II) was purified *via* its oxalate (m.p. 205° decomp., crystallised from dilute alcohol) and picrate, m.p. 185-88° (decomp.). The base had m.p. 170° (decomp.). (Found : C, 48.7, 48 4 ; H, 6.0, 6.1 ; N, 20.7. $C_8H_{13}O_3N_3$ requires C, 48.3 ; H, 6.5; N, 21 1 per cent). The I R. spectra (Fig. 4) show the following :

| Bands. | Functional groups. | Bands. | Functional groups. |
|---|---|---|---|
| 2.9 $\mu$ | Bonded NH | 6 6 $\mu$ | $NO_2$ |
| 5.8 | CO-NH | 7.25 | C-$CH_3$ |
| 5.95 | | 7 8 | Unidentified |
| 6.3 | Conjugated double bond | | |

The substance then probably is



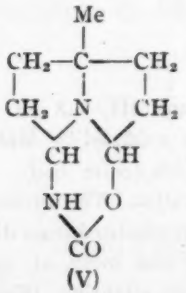
*Oxidation of* (II) *by* $MnO_2$.—The compound (II, 0.8 g.) was dissolved in water (20 c.c.) containing $H_2SO_4$ (1.5 c.c.) and was oxidised by $MnO_2$ (2 g.) added in small portions during 1 hour, the solution being kept on gentle boil. On standing, a sticky substance separated which was removed by filtration. The filtrate on standing a few days deposited a crystalline substance which was crystallised from dilute alcohol containing a few drops of acetic acid. It is an acid and melts at 190-92° (decomp.). The substance (IV) is difficult to dry as it decomposes slightly. (Found : C, 40.2 ; H 6.3 ; N, 17.0. Calc. for $C_6H_{10}O_4N_2$ : C, 41.3 ; H, 5.7 ; N, 16.1 per cent). The substance on the basis of formula for chaksine is represented by structure (IV).



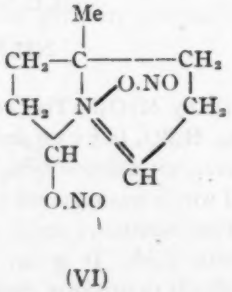
(IV)

*Action of Nitrous Acid on Chaksine.*—The interaction of 1 mole of $HNO_2$ on 1 mole of chloride afforded a substance, m.p. 221°, which Ray *et al.* (*loc. cit.*) erroneously formulated as $C_9H_{17}O_4N_3$. This substance has now been found to be chaksine nitrite and not nitrate, as suggested by Gupta and Mahajan (*J. Sci. Ind. Res.*, 1955, **14B**, 602). The formation of a nitrate by interaction with nitrous acid would be most remarkable.

Chaksine sulphate (5.48 g.) was converted into the chloride by interaction with a solution of $BaCl_2$, $2H_2O$ and cooled to 0°. A solution of sodium nitrite (9.0 g.) in water (100 c.c.), cooled to 0°, was mixed with a solution of HCl (*d* 1.16, 19 c.c.) in water (100 c.c.), also cooled to 0°. To this was added the cooled chaksine chloride solution during 1½ hours at 0°. The mixture was left at 25°-30° for 3 hours. After that, the solution was made ammoniacal when a precipitate appeared. This was dissolved in ethyl acetate, dried and from the solution the substance was recovered. The residue gave a highly crystalline oxalate in alcohol, m.p. 298° (decomp.) after several recrystallisations from alcohol (V). The ammoniacal aqueous solution was just acidified with acetic acid, when silky needles separated. These were collected and crystallised successively from hot water, and a mixture of alcohol and ether (VI). The aqueous filtrate on slow evaporation provided a further crop of this substance. It had m.p. 210° (decomp.). It is a quaternary salt; after treatment with alkali in the aqueous alkaline solution strong prositive test for a nitrite is obtained. [Found in (V): C, 48.5; H, 6.6; N, 11.8. $C_{20}H_{34}O_{16}N_4$ requires C, 49.0; H, 6.9; N, 11.4 per cent]. Therefore (V) is the oxalate of the base $C_9H_{16}O_3N_2$. [Found in (VI): C, 44.1; H, 6.9; H, 6.2; N, 19.5]. The substances are oxalate of



(V)



(VI)

*Action of Fuming Nitric Acid on Chaksine Sulphate.*—Chaksine sulphate (7.0 g.) was added to fuming nitric acid (95%, 28 c.c.) at 30° in small quantities

with shaking. After standing for 18 hours, the mixture was poured into ice cold water (200 c.c.). A yellow precipitate was obtained, which was collected, washed with water and dried (A). The filtrate was designated as (B).

*Treatment of A.*

A

Treated with $NaHCO_3$ soln.

Soluble (A-1) precipitated by HCl

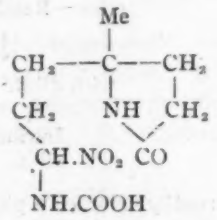
Insoluble

Treated with $Na_2CO_3$

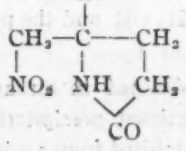
Soluble (A-2) precipitated by HCl

Insoluble (A-3)

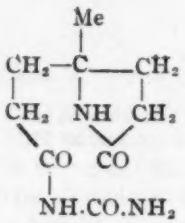
The substance (A-1) is soluble in alcohol, acetic acid and acetone. It gave an anilide, m.p. 208° but it could not be recrystallised. The substance (A-1) was suspended in cold when a sticky substance remained insoluble. The alcoholic filtrate was treated with an alcoholic solution of lead acetate when its lead salt was precipitated. The substance was obtained in a crystalline condition from its lead salt and had m.p. 180° (decomp.). The sticky liquid, insoluble in cold alcohol, was cooled in ice and washed with ice-cold water when it solidified. It was crystallised from excess of alcohol avoiding too much heating of the solvent as it decomposed in contact with hot solvent. The substance (fine needles) had m.p. 80° (decomp.). (Found: C, 43.5; H, 6.5; N, 17.5. Calc. for $C_9H_{15}O_5N_3$: C, 43.9; H, 6.1; N, 17.2 per cent). The substance contains a nitro group and is an acid. It is formulated as



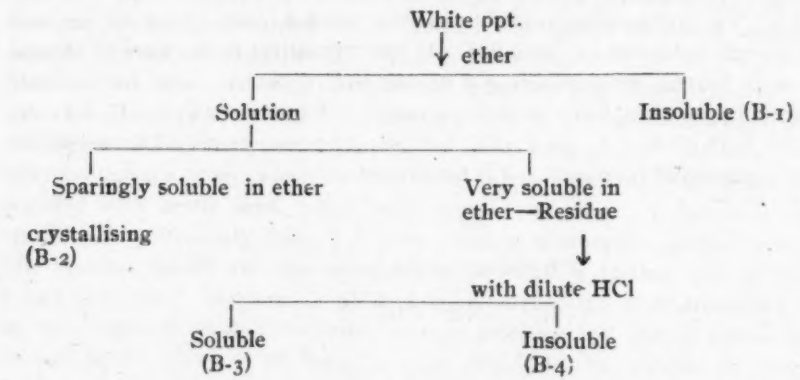
The substance (A-2) was purified by repeated crystallisation from dilute alcohol and had m.p 145° (shrinkage earlier). It was then kept in contact with ether for 24 hours when the impurities dissolved. The residue was again crystallised from dilute alcohol when it had m.p. 160° (decomp.). (Found: C, 45.1; H, 6.7; N, 18.2. $C_6H_{10}O_5N_2$ requires C, 45.5; H, 6.3; N, 17.7 per cent). The substance contains a $NO_2$ group and has NH-CO- group. It furnished an N-Ag salt. It is formulated as



*A*-3.—This substance dissolves in dilute sodium hydroxide solution from which it is reprecipitated by acids. It was purified by conversion into the picrate, which was purified by recrystallisation from acetone-alcohol mixture, m.p. 199° (decomp.). [Found: C, 41.1; H, 4.4; N, 19.2. $C_{18}H_{18}O_{18}N_6$ (*i.e.* picrate of $C_9H_{18}O_3N_3$) requires C, 40.7; H, 4.1; N, 19.0 per cent). The substance contains $CO\text{-}NH_2$ group as it liberates $NH_3$ on heating with alkali. It has the structure




The filtrate *B* was made slightly alkaline with $NaHCO_3$ when a flocculent precipitate separated. This precipitate was treated as follows.



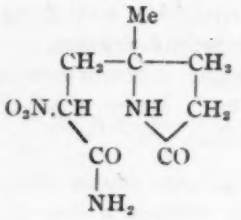

*B*-1, formed in small yield, readily afforded a picrate (deep yellow needles), m.p. 202° (decomp.) after crystallisation from absolute alcohol. (Found: C, 40.8; H, 4.2; N, 19.6. $C_{17}H_{21}O_{11}N_7$ requires C, 40.9; H, 4.2; N, 19.6 per cent). It is the picrate of chaksine of which the $CH_2.OH$ has formed a nitric ester, *i.e.* $CH_2.O.NO_2$. The substance responds to the test for a nitrate.

*B*-2 is sparingly soluble in ether and separates out during the distillation of the ethereal solution. It was repeatedly crystallised from alcohol in beautiful needles, m.p. 174-78° (decomp.). (Found: C, 48.7; H, 6.7; N, 20.6. $C_{11}H_{18}O_4N_4$ requires C, 48.9; H, 6.7; N, 20.7 per cent). M.W. (cryoscopic in acetic acid), 266. This substance is the nitric ester at $CH_2OH$ and the pentavalent N is in the aminic oxide form, *i.e.* $:N=O$ form.

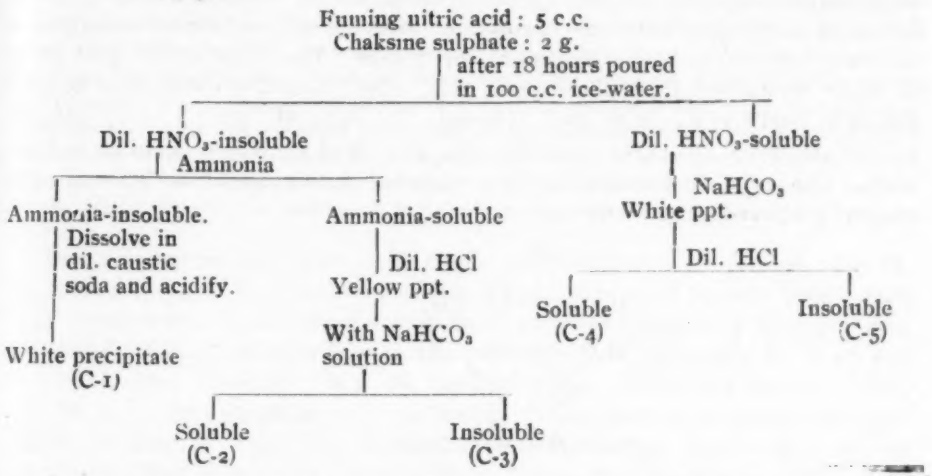
The substance B-3 was not obtained in an analytically pure condition.

*B*-4 was initially purified by fractional precipitation of its alcoholic solution with petroleum ether. Finally, it was crystallised from a mixture of alcohol and petroleum ether

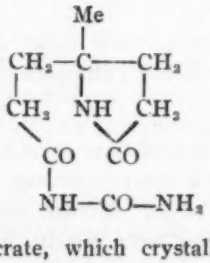
when it had m.p. 130°. (Found: C, 41.0; H, 6.9; N, 18.2. Calc for $C_8H_{15}O_5N_3$: C, 41.1; H, 6.4; N, 18.0 per cent). It is represented as shown below.



In another experiment the ratio of chaksine sulphate to fuming nitric acid was altered to 2 g. of chaksine to 5 c.c. of fuming nitric acid at 20°. The products were isolated as per scheme below. The products C-1, C-2 and C-4 were formed in fairly good yields.



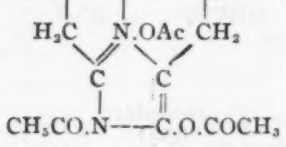

*C*-1 is insoluble in sodium bicarbonate, sodium carbonate and ammonia. It readily dissolves in sodium hydroxide solution. It was crystallised from acetic acid, m.p. 266° (decomp.). It was analysed after drying in vacuo at 180°. (Found: C, 49.5; H, 7.25; N, 19.7. $C_9H_{15}O_5N_3$ requires C, 50.5; H, 7.3; N, 19.7 per cent). It is represented as



*C*-4 was purified as its picrate, which crystallsed from a mixture of alcohol and acetone, m.p. 199° (decomp.). [Found: C, 42.2; H, 4.5; N, 19.3. $C_{15}H_{20}O_9N_6$ (*i.e.* picrate of $C_9H_{17}N_2O_2$) requires C, 42.1; H, 4.7; N, 19.6 per cent]. This substance has the same formula as C-1, except that in ring NH-CO is NH-$CH_2$ in this compound. The substance furnishes chloroplatinate, m.p. 210° (decomp.) whence its M.W. is found to agree with the structure proposed. The other products were not found to be analytically pure.

*Benzoylation of Chaksine.*—Chaksine chloride (5.0 g.), dissolved in water (50 c.c.), was benzoylated with benzoyl chloride (10 c.c.) and alkali. After standing overnight, the benzoyl derivative solidified and was repeatedly crystallised from alcohol, m.p. 140°. Siddiqui *et al.* (*loc. cit.*) reported two benzoyl derivatives, m.p. 273° and m.p. 146-52°. Our substance, m.p. 140°, is a simple dibenzoylbenzoate of chaksine (one benzoyl at $CH_2.OH$ and the other at one of the N atoms in NH-CO-NH of chaksine; the third as the benzoate at the quaternary N atom).

*Acetylation of Chaksine.*—Chaksine sulphate (1.0 g.) was heated with acetic anhydride (10 c.c.) and pyridine (3 drops) in a boiling water-bath for 2 hours. Excess of acetic anhydride was removed in *vacuo* at 80°. The residue was poured into water (100 c.c.) and allowed to stand for 2 days. The white sticky solid could be easily crystallised from dilute acetic acid, dilute alcohol or a mixture of benzene and ether, yield 0.25 g., m.p. 200°. [Found: C, 58.1; H, 7.1; N, 9.2; $CH_3CO$, 41.0. Calc. for $C_{15}H_{20}O_5N_2$ (triacetyl): C, 58.4; H, 6.5; N, 9.1; $CH_3CO$, 41.6 per cent]. The amino-ethanol chain has been detached during drastic acetylation. The triacetyl compound is represented as




*Oxidation with Chromic Acid.*—Chaksine sulphate (5.48 g.), suspended in water (50 c.c.), was heated under reflux and the mixture was oxidised with a small amount of $CrO_3$ (4 g. in 2*N*-$H_2SO_4$, 40 c.c.) during 4 hours. Carbon dioxide was freely evolved. The solution on standing overnight gave a white crystalline deposit, which was repeatedly crystallised from hot very dilute sulphuric acid, m.p. 310° (decomp.). [Found: C, 39.4; H, 5.5; N, 14.6; S, 5.6. $(C_9H_{15}O_4N_3)_2SO_4$ requires C, 39.0; H, 5.4; N, 15.1; S, 5.8 per cent]. The exact formulation of the compound has not been possible. It is probably a mixture although it looks quite homogeneous under the microscope.

Emde degration, Hoffmann degradation, zinc dust distillation, selenium dehydrogenation, soda-lime distillation did not provide any recognisable products. Chaksine sulphate and potassium hydroxide at 300° afforded only ammonia and potassium cyanide as recognisable products. Oxidation with NaOBr furnished bromoform as the only characterisable product.

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# HEATS OF WETTING OF POROUS BODIES BY WATER AND METHYL ALCOHOL

By Balwant Rai Puri, Romesh Datt and Lekh Raj Sharma

Heats of wetting by water and methyl alcohol were determined in the case of three samples of charcoal, a sample of bentonite and a sample each of silica, alumina, ferric oxide and chromic oxide gels before as well as after associating them with varying amounts of water and methyl alcohol. The thermal effect in the case of dry adsorbents was more with methyl alcohol than with water, and for a given liquid was found to depend upon the surface area of the adsorbent which, in fact, could be thus estimated. The measurement of heats of wetting of the solid as a function of the amounts of liquids previously adsorbed permitted calculations of heats of adsorption of monolayers of liquid (from the liquid phase) and isosteric heats of adsorption. There is no relationship between the heat of adsorption of a liquid in the monolayer and its heat of fusion, as pointed out by previous workers. Isosteric heats of adsorption are positive and tend to drop to zero after the completion of the monolayer.

In a previous communication (Puri, Gupta and Lakhanpal, this *Journal*, 1954, **31**, 435) it has been shown that heat of immersion of charcoal in water depends largely on its water isotherm as a whole and not on any single point on the graph. Now, since the adsorption isotherm itself depends on surface area of the solid, as shown by Boyd and Harkins (*J. Amer. Chem. Soc.*, 1942, **64**, 1190) and Kistler, Fischer and Freeman (*ibid.*, 1943, **65**, 1909), there should be some relationship between heat of immersion of a dry solid in a wetting liquid and its surface area. Some investigators have noted a sort of dependence of heat of immersion on particle size (Parkes, *Phil. Mag.*, 1902, *vi*, **4**, 240; Iliin *et al.*, *ibid.*, 1937, *vii*, **23**, 294) or even surface area (Iliin, Kiselev, Likhachava and Scherhakova, *Doklady Akad. Nauk.*, S.S.S.R., 1950, **75**, 827), but the exact relationship between these quantities does not appear to have been worked out and the possibility of determining surface area of powders by this simple technique has not been explored.

Stowe (*J. Phys. Chem.*, 1952, **56**, 487) and Puri *et al.* (*loc. cit*), from measurements of the heats of immersion as a function of the amount of water pre-adsorbed on the solid, have shown that the heat of formation of a monolayer of water (derived from liquid water), in the case of alumina gels and charcoal, is nearly twice the heat of fusion of ice. This relationship, however, needs verification by carrying out similar investigations on other solid-liquid systems as well.

By combining heats of immersion data with the results obtained from an adsorption isotherm at one temperature, it is possible to calculate isosteric heats of adsorption (Jura and Hill, *J. Amer. Chem. Soc.*, 1952, **74**, 1598). This procedure was utilised by Young *et al.* (*J. Phys. Chem.*, 1954, **58**, 313) in calculating isosteric heats of adsorption of water on graphon, which was found to be negative. It will be of interest to make similar calculations in the case of porous adsorbents which have large areas and high adsorption capacities.

The work described in this paper was undertaken with these objectives in view.

## Experimental

Three samples of charcoal (prepared by the carbonisation of cane sugar, cocoanut shell and pine wood), a sample of bentonite, and gels of silica, alumina, ferric oxide and chromic oxide, prepared by the usual precipitation methods and dried to constant weights at 110°, were used in these investigations. Water (redistilled) and methyl alcohol (Merck's extra pure, free from acetone) were used as the wetting liquids. Heats of wetting of the various samples, when dry and when different amounts of water (or methyl alcohol) had been pre-adsorbed on them in equilibrium with atmospheres of different relative vapour pressures at 25° (Puri, Lakhanpal and Verma, this *Journal*, 1952, **29**, 841), were determined in water as well as in methyl alcohol by using the same technique as described in the previous communication (*loc. cit.*). Surface areas of the various samples were calculated from their water and methyl alcohol isotherms by following Boyd and Harkins' calculations (*loc. cit.*) and a mean value was taken in each case.

## Discussion

*Heat of Wetting and Surface Area.*—The heats of wetting of the oven-dried samples in water and methyl alcohol are shown in Table I. It is seen that in every case more heat is evolved in methyl alcohol than in water. The adsorbability of methyl alcohol was also found to be greater than that of water, particularly in the lower parts of the isotherms. This may explain higher thermal values in methyl alcohol.

The thermal values, expressed in terms of ergs/cm$^2$. are also included in Table I.

Table I

*Heats of wetting of the dry adsorbents by water and methyl alcohol.*

| Adsorbent. | Surface area ($m_s$/g.). | Heat of wetting (cal./g.) by | | Heat of wetting (ergs./cm$^2$) by | |
|---|---|---|---|---|---|
| | | Water. | MeOH. | Water. | MeOH. |
| Alumina gel | 236.6 | 17.93 | 19.50 | 318.2 | 345.3 |
| Silica gel | 339.7 | 25.75 | 38.25 | 318.8 | 440.4 |
| Chromic oxide gel | 150.3 | 11.02 | 15.67 | 308 6 | 438.6 |
| Ferric oxide gel | 204.9 | 14.50 | 19.83 | 296.1 | 396.7 |
| Bentonite | 138.8 | 9.95 | 11.85 | 301.4 | 358.1 |
| Sugar charcoal | 180.5 | 12.30 | 18 91 | 290.6 | 440.1 |
| Pine charcoal | 123.4 | 9.18 | 13.82 | 312.7 | 433.3 |
| Cocoanut charcoal | 139.1 | 9.59 | 14.88 | 289.9 | 449.9 |

It is interesting to note that the values in a given liquid, particularly in water, are fairly close to one another, irrespective of the surface area or the nature of the adsorbent. This shows that the heat of immersion is largely a surface phenomenon and that the surfaces involved in the experiments are all alike. An average value of 305 ergs may be taken for wetting 1 sq. cm. of surface in water. This is quite significant since it enables one to calculate surface areas of porous bodies from a single value, namely, heat of wetting in water. In order to verify this a few more samples of charcoal, soils and gels (prepared by different methods) were taken. Their water isotherms as well as heats of immersion in water were determined. The surface areas calculated from the isotherms by Boyd and Harkins' method and from the heats of wetting are compared in Table II. The agreement is seen to be as good as could be expected.

This places at our disposal quite a convenient method for estimating surface areas of porous bodies.

TABLE II

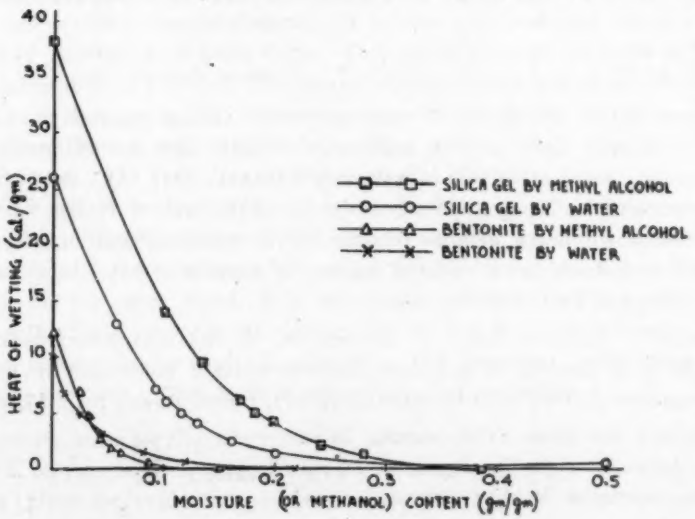
*Comparison of surface areas determined from heats of wetting in water and from water isotherms by Harkin's method.*

| Adsorbent. | Surface area ($m^2/g.$) calc. from | | Adsorbent. | Surface area ($m^2/g.$) calc. from | |
|---|---|---|---|---|---|
| | Heat of wetting. | Water isotherm. | | Heat of wetting. | Water isotherm. |
| Alumina (from potash alum) | 259 7 | 280.6 | Ferric oxide (from ferric alum precipitated at 100°) | 159.7 | 140.8 |
| Alumina (from aluminium amalgam) | 231.9 | 242.7 | Sugar charcoal (activated) | 210.6 | 241.6 |
| Alumina (from aluminium butoxide) | 212.3 | 218.2 | Acacia wood charcoal | 124.4 | 164.7 |
| Chromic oxide (from chrome alum) | 142.7 | 160.7 | Cotton stalk charcoal | 118.1 | 142.5 |
| Chromic oxide (from chrome alum precipitated at 100°) | 139.9 | 126.2 | α-Stannic acid | 101.5 | 89.98 |
| | | | β-Stannic acid | 121.7 | 109.4 |
| Ferric oxide (from ferric alum) | 198.9 | 232.1 | Soil P.C. 13 | 149.8 | 150.8 |
| | | | Soil P C. 19 | 106.9 | 88.9 |

*Calculation of Heat of Adsorption of a Monolayer (from liquid phase).*—The heat effects on immersing silica gel and bentonite with different surface coverage in water and methyl alcohol are shown graphically in Fig. 1. The other adsorbents showed similar results (not included for reasons of space). It is evident (Fig. 1) that the heat of immersion of a solid decreases appreciably when it contains even a small amount of the liquid pre-adsorbed on it.

FIG. 1

*Heat of wetting as a function of the amount of the liquid previously adsorbed.*



The amounts of water and methyl alcohol required to form monolayers in the case of the various adsorbents were calculated, assuming that a molecule of water covers 10$A^2$ and that of methyl alcohol 16 $A^2$ (Brunauer, "Adsorption of Gases and Vapours", Vol. I, 1942, Princeton Univ. Press). The heats of wetting of the various samples, when they already contained these amounts of the liquids, were read from the respective graphs, and subtracting these from the heats of wetting of the fresh samples, the heats of adsorption of monolayers (from liquid phase) were calculated. Dividing these by the weights of water (or methyl alcohol) in the monolayers, the amounts of heat per gram of water (or methyl alcohol) adsorbed in the monolayer could be obtained. For example, in the case of silica gel (surface area = 339.7 $m^2$/g.) the amounts of water and methyl alcohol required to form monolayers are 0.1017 g. and 0.1132 g. respectively. Bare silica gel evolves 25.75 and 38.25 cal./g. adsorbent when wetted by water and methyl alcohol respectively. From the curves plotted in Fig. 1 for the silica gel it is estimated that one gram of the gel, on which 0.1017 g. of water and 0.1132 g. of methyl alcohol are already adsorbed, would evolve 6.50 and 12.50 cal. on wetting with water and methyl alcohol respectively. Hence, to cause a monolayer of water and methyl alcohol to be adsorbed (from the liquid phase) on the silica gel, the evolution of 19.25 and 25.75 cal./g. of the adsorbent or 189.6 and 227.5 cal./g. of water and methyl alcohol respectively would result.

The results obtained in case of the various adsorbents are shown in Table III.

TABLE III

*Heat of wetting by water and methyl alcohol in the formation of monolayer.*

| Adsorbent. | Heat produced of liquid in the formation of monolayer by | | Adsorbent. | Heat produced of liquid in the formation of monolayer by | |
|---|---|---|---|---|---|
| | Water. | MeOH. | | Water. | MeOH. |
| Alumina gel | 181.4 cal./g. | 207.0 cal./g. | Bentonite | 157.4 cal./g. | 200.6 cal./g. |
| Silica gel | 189.6 | 227.5 | Sugar charcoal | 167.3 | 215.6 |
| Chromic oxide gel | 173.6 | 219.7 | Pine charcoal | 190.0 | 231.2 |
| Ferric oxide gel | 179.2 | 213.4 | Cocoanut charcoal | 179.9 | 221.3 |

It is seen that when a monolayer of water or methyl alcohol is added, the heat evolved per gram of a given liquid is of the same order in all the samples examined. Further, while the average value in the case of water is a little more than twice the heat of fusion, in the case of methyl alcohol it is nearly seven times the heat of fusion. It is evident therefore that there is no definite relationship between the heat of adsorption of a liquid in the monolayer and its heat of fusion, as appears to have been suggested by Stowe (*loc. cit.*) and Puri *et al.* (*loc. cit.*).

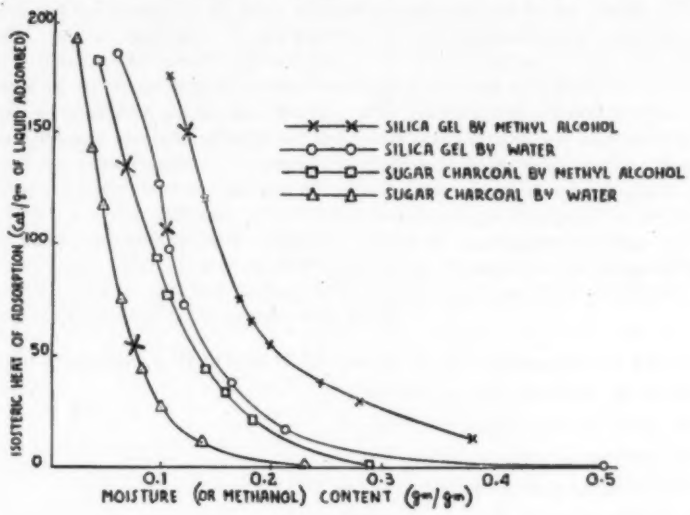
*Calculation of Isosteric Heats of Adsorption.*—It has been shown by Young *et al.* (*loc. cit.*) that it is possible to calculate isosteric heats of adsorption by means of the following equation derived from the work of Hill (*J. Chem. Phys.*, 1949, **17**, 520).

$$U_o - U = \int_0^{N_s} q_{st.} dN_s - \Delta H_v ,$$

where $U_o - U$ is the difference in the heat of immersion of the clean solid and a solid with $N_s$ adsorbed molecules and $\Delta H_v$ is the molar heat of vaporisation of the liquid. The isosteric heat is thus obtained by a graphical differentiation of $U_o - U$ *versus* $N_s$ plot.

FIG. 2

*Isosteric heats of adsorption as a function of the amount of the liquid adsorbed.*




The isosteric heat values calculated in this manner are plotted as a function of the amount of water (or methyl alcohol) adsorbed in Fig. 2 in the case of silica gel and sugar charcoal. Similar curves were obtained in the case of the other adsorbents but are not included for reasons of space. It is seen that the heat effect is positive at all stages of adsorption in every case. This observation is of particular interest in the case of adsorption of water on charcoal on account of the fact that several workers have reported both positive and negative heats of adsorption of water on various types of carbon (Bartell and Suggitt, *J. Phys. Chem.*, 1954, **58**, 36 ; Pierce and Smith, *ibid.*, 1948, **52**, 1111, 1115 ; Pierce *et al.*, *J. Amer. Chem. Soc.*, 1951, **73**, 455 ; Anderson and Emmett, *J. Phys. Chem.*, 1952, **56**, 753). The discrepancies were probably due to different nature of the carbon surfaces examined by them. If a surface is easily wetted by water, *i.e.*, contact angle is zero, the heat effect will be positive. If, on the other hand, it is not easily wetted, *i.e.*, a contact angle is formed, the adhesion of water to the surface will be less than the cohesion of water. Therefore in order to cause water to adhere to the adsorbent rather than to itself energy must be provided. This leads to negative heat of adsorption.

The isosteric heats for the adsorption of monolayers of water and methyl alcohol are marked X on the respective graphs for the silica gel and sugar charcoal, plotted in Fig. 2. The values are evidently positive (*i.e.* the total heats of adsorption are greater

than the corresponding heats of liquefaction) but they tend to drop to zero (*i.e.*, the total heats of adsorption tend to approach the corresponding heats of liquefaction) as second and subsequent statistical surface layers are formed. These observations are in accordance with the requirements of the BET theory.

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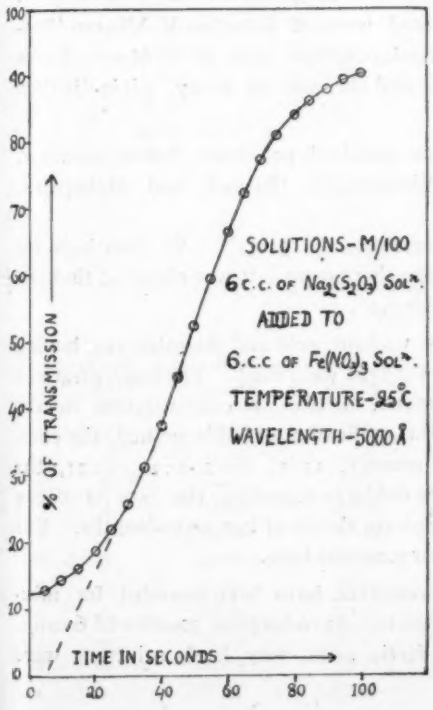
# SPECTROPHOTOMETRIC STUDY OF THE FERRIC-THIOSULPHATE COMPLEX. PART I

By J. Das and D. Patnaik

Determination of the formula of the labile coloured complex, formed by the reaction of thiosulphates with ferric salts, by the application of Job's method of continuous variation to the optical properties of the coloured complex has been reinvestigated due to the special nature of the decay curve. Three aqueous solutions of two ferric salts, one without acid and two others with different acid contents, have been used at two wave-lengths, 500 mμ and 600 mμ. The maximum absorption for the volume ratio of 1 :1 of equimolecular solutions of ferric salt and thiosulphate without any acid confirms the formula $Fe^{+}SO_3$. The volume ratio changed to 1 :2 and 1 :3 according to the different acid contents. This has been explained as due to the difference between the effective concentration and the actual concentration of $S_2O_3^{2-}$ ion. When acidic solutions of ferric salts were used, the absorptions at zero-time for wave-lengths 500 mμ and 600 mμ were not much different. But with solutions containing no acid, the difference was very high. The increase in absorption in case of aqueous solutions containing no acid at lower wave-length region is ascribed to the polynuclear hydrolysis product $Fe(OH)_2Fe^{4+}$. Indication for the existence of $Fe(OH)_3Fe^{2+}(S_2O_3)$ has also been given.

A coloured intermediate is produced by the reaction of thiosulphates with ferric salts. The curve representing the rate of decay of this coloured intermediate, as obtained by the plot of the percentage of transmission against time, was found to be of S-shape (Patnaik and Mahapatra, *Curr. Sci.*, 1955, **24**, 195). This is contrary to the observations of Haldar and Banerjee (*Proc. Nat. Inst. Sci.*, 1948, **14**, 1) and that of Page (*Trans. Faraday Soc.*, 1953, **49**, 635) who used optical methods for assigning the formula $Fe^{+}S_2O_3$ to this coloured complex. For the application of Job's method of continuous variation (*Ann. chim.*, 1928, *x*, **9**, 113) to the determination of the formula of the coloured complex, the absorption due to the complex just at the moment of mixing the solutions of ferric salt and thiosulphate has to be evaluated. This is done by extrapolation of the decay curve to zero-time. Since the extrapolation value depends solely on the nature of the curve, our S-shaped curves are bound to provide different values from those of Haldar and Banerjee, and of Page. It can be seen from Fig. 1, that any endeavour to extrapolate the curve, neglecting the first

FIG. 1



few points up to the point of inflection, would give rise to negative transmission which cannot be accepted because of the fact that the solution cannot have negative transmission value at zero-time. Thus, reinvestigation of the application of the Job's method to the determination of the formula spectrophotometrically became necessary.

## EXPERIMENTAL

Unicam diffraction-grating spectrophotometer (Model 350) was used. The wavelength was calibrated by the dyodinium-filter supplied with the instrument. Particular care was taken to see that the intensity of the light beam did not change during the course of a run which was of not more than a few minutes' duration, mostly 100 seconds. The checking was done by comparing at the end of each run with the standard cell containing water for 100% transparency.

Quick-delivery pipettes were specially prepared so that the time of delivery of the solution did not last for more than 1.3 seconds. Stirring by hand was carried with a special stirrer for 2 to 3 seconds. At the end of the 5th second readings were taken. The reliability of the first reading at the 5th second was verified by a dichromate solution for 3 seconds and then noting the % transmission at the end of the 5th second, which was the same as the one taken without stirring. All the cells used were calibrated to porvide the same transparency with standard one.

Ferric salts and sodium thiosulphate were of E. Merck Extra Pure quality or B.D.H. Analar quality. Hydrochloric and nitric acids used were of Schering-Kahlbaum 'Pro-analysi' quality. All other reagents used for standardisation were of E. Merck Extra Pure quality. Since copper contamination accelerated the rate of decay, triple-distilled water in all-pyrex distillation set was used.

Thiosulphate solution was standardised against standard potassium iodate solution. Solutions of ferric salts were standardised iodimetrically (Patnaik and Mahapatra, unpublished).

A thermostat was maintained at 25° with fluctuation of ± 0.1°. All solutions for use were kept in stoppered Erlenmeyer flasks in the thermostat. It was observed that the rise of temperature for a run lasting 3 minutes was 0.2°.

Three solutions of ferric salts in water, one without acid and the other two having corresponding acid concentrations of 0.1*M* and 0.25*M* were used. The concentrations of $Fe^{3+}$ and of $S_2O_3^{2-}$ in their respective solutions (not the effective concentration in the mixture) were each of 0.01*M* strength. For the application of Job's method, the composition of the mixture was varied in the manner, 11:1, 10:2,-2:10, 1:11, the total volume being always 12 c.c. In Table I the readings recording the rate of decay for a set of three mixtures of different compositions are shown at two wave-lengths. For economy of space 110 such readings have not been recorded here.

In Tables II and III the % transmissions at zero-time have been recorded for mixtures of different compositions of the two ions at the two wave-lengths, 500mμ and 600mμ. Due to rapid hydrolysis of aqueous solutions of ferric salts, very fresh solutions were used for experiments.

## TABLE I

Temperature = 25° ± 0.1°.

| Time. | 6 c.c. of 0.01*M*-$Na_2S_2O_3$ soln. added to 6 c.c. of 0.01*M*-$Fe(NO_3)_3$ soln. without acid. | | 8 c.c. of 0.01*M*-$Na_2S_2O_3$ soln added to 4 c.c. of 0.01*M*-$Fe(NO_3)_3$ soln. containing 0.1*M*-$HNO_3$. | | 9 c.c. of 0.01*M*-$Na_2S_2O_3$ soln. added to 3 c.c. of 0.01*M*-$Fe(NO_3)_3$ soln. containing 0.25*M*-$HNO_3$. | |
|---|---|---|---|---|---|---|
| | % Transmission at | | % Transmission at | | % Transmission at | |
| | 500 mμ. | 600 mμ. | 500 mμ. | 600 mμ. | 500 mμ. | 600 mμ. |
| 5 sec. | 13.00 | 26.25 | 21.50 | 23.00 | 38.00 | 39.50 |
| 10 | 14.50 | 28.50 | 24.25 | 25.75 | 39.75 | 41.00 |
| 15 | 16.25 | 30.75 | 28.00 | 29.75 | 43.25 | 43.25 |
| 20 | 18.75 | 34.00 | 32.75 | 35.25 | 46.00 | 45.75 |
| 25 | 22.00 | 37.75 | 39.25 | 42.25 | 48.75 | 48.50 |
| 30 | 25.75 | 43.25 | 47.00 | 49.75 | 51.25 | 51.50 |
| 35 | 31.50 | 48.75 | 54.25 | 57.75 | 56.75 | 54.25 |
| 40 | 37.50 | 54.75 | 61.75 | 64.50 | 59.50 | 57.00 |
| 45 | 44.75 | 61.25 | 68.00 | 70.50 | 62.00 | 60.00 |
| 50 | 52.50 | 67.50 | 73.25 | 75.50 | 64.50 | 62.75 |
| 55 | 59.50 | 73.50 | 77.75 | 79.75 | 66.00 | 65.50 |
| 60 | 66.50 | 79.00 | 81.25 | 83.00 | 69.00 | 67.75 |
| 65 | 72.50 | 83.50 | 83.50 | 85.25 | 70.75 | 70.25 |
| 70 | 77.50 | 86.75 | 86.00 | 87.25 | 72.75 | 72.25 |
| 75 | 81.25 | 89.50 | 88.00 | 88.75 | 74.50 | 74.50 |
| 80 | 84.25 | 91.50 | 89.00 | 90.25 | 75.75 | 76.50 |
| 85 | 86.25 | 92.50 | 90.00 | 91.25 | 77.50 | 77.75 |
| 90 | 88.00 | 94.00 | 91.00 | 92.25 | 78.50 | 79.25 |
| 95 | 89.50 | 94.50 | 92.00 | 92.75 | 80.00 | 80.50 |
| 100 | 90.25 | ... | 92.75 | 93.50 | 80.75 | 81.50 |

## TABLE II

% *Transmission at zero-time of* $Fe(NO_3)_3$ *solution.*

Temp. = 25° ± 0.1°.

| Comp. of soln. (c.c.) $Fe^{3+}$ : $S_2O_3^{2-}$. | | Fe solns. without acid. | | Fe solns with 0.1*M*-$HNO_3$. | | Fe solns. with 0.25*M*-$HNO_3$. | |
|---|---|---|---|---|---|---|---|
| | | 500 mμ. | 600 mμ. | 500 mμ. | 600 mμ. | 500 mμ. | 600 mμ. |
| 11 | 1 | 48.50 | 68.00 | 76.50 | 77.75 | 89.00 | 89.00 |
| 10 | 2 | 31.00 | 49.75 | 59.75 | 62.00 | 79.00 | 81.00 |
| 9 | 3 | 21.25 | 37.00 | 46.75 | 49.00 | 69.50 | 71.50 |
| 8 | 4 | 15.75 | 30.00 | 38.00 | 39.25 | 62.00 | 63.50 |
| 7 | 5 | 13.00 | 26.25 | 29.00 | 31.50 | 54.50 | 56.00 |
| 6 | 6 | 12.25 | 25.25 | 25.75 | 26.50 | 48.75 | 49.50 |
| 5 | 7 | 15.00 | 26.75 | 22.25 | 22.50 | 43.25 | 45.00 |
| 4 | 8 | 18.00 | 32.00 | 20.25 | 21.50 | 39.00 | 40.20 |
| 3 | 9 | 27.25 | 42.50 | 22.50 | 23.50 | 31.00 | 38.25 |
| 2 | 10 | 40.25 | 55.75 | 29.00 | 31.00 | 38.50 | 39.50 |
| 1 | 11 | 66.25 | 77.50 | 49.50 | 51.00 | 52.00 | 53.50 |

TABLE III

*% Transmission at zero-time of* $FeCl_3$ *solution.*

Temp. = 25° ± 0.1°.

| Comp. of soln (c.c.) | | Fe solns. without acid. | | Fe solns. with 0.1*M*-HCl. | | Fe solns. with 0.25*M*-HCl. | |
|---|---|---|---|---|---|---|---|
| $Fe^{3+}$ : | $S_2O_3^{2-}$ | 500 mμ. | 600 mμ. | 500 mμ. | 600 mμ. | 500 mμ. | 600 mμ. |
| 11 | 1 | 50.75 | 68.50 | 78.50 | 81.75 | 88.50 | 92.00 |
| 10 | 2 | 32.25 | 50.00 | 64.75 | 67.00 | 81.75 | 85.00 |
| 9 | 3 | 22.75 | 37.50 | 52.50 | 56.00 | 75.75 | 78.25 |
| 8 | 4 | 17.50 | 31.50 | 44.00 | 46.75 | 69.00 | 71.25 |
| 7 | 5 | 15.00 | 27.25 | 35.50 | 37.75 | 61.75 | 64.50 |
| 6 | 6 | 14.50 | 26.00 | 30.50 | 32.50 | 56.25 | 59.25 |
| 5 | 7 | 16.50 | 28.50 | 26.75 | 28.25 | 50.25 | 53.25 |
| 4 | 8 | 20.25 | 33.25 | 24.25 | 27.00 | 46.00 | 48.25 |
| 3 | 9 | 27.75 | 42.50 | 26.50 | 28.25 | 43.25 | 45.25 |
| 2 | 10 | 41.25 | 57.00 | 33.25 | 35.00 | 44.00 | 46.00 |
| 1 | 11 | 66.75 | 78.50 | 52.25 | 53.75 | 55.50 | 55.75 |

## DISCUSSION

From Tables II and III the % transmission at both the wave-lengths appears to be minimum for the mixture of equal composition when solutions containing no acid of both ferric salt and aqueous solution of thiosulphate are used. This suggests the formula $Fe^+S_2O_3$ for the coloured complex and confirms the observation of Haldar and Banerjee (*loc. cit.*). In case of the solutions of ferric salts containing 0.1*M* acid, the minimum was found for a mixture of 4 c.c. of ferric salt solution and 8 c.c. of thiosulphate solution. For acid content of 0.25*M* in the solution of ferric salts, the minimum was observed for the ratio of 3:9. The experiments with acid solutions would ordinarily suggest such formulae as $Fe(S_2O_3)_2^-$ and $Fe(S_2O_3)_3^{3-}$ for the coloured complex under different $H^+$ concentrations. In aqueous solution of sodium thiosulphate, except for the ion pair $NaS_2O_3^-$ present to a very small extent, almost the whole of $S_2O_3^{2-}$ ions are available for formation of the complex according to the following reversible reaction, suggested by Schmid (*Z. physikal. Chem.*, 1930, **148**, 321):

$$Fe^{3+} + S_2O_3^{2-} \rightleftharpoons Fe^+S_2O_3 \quad \ldots \quad \ldots \quad (1)$$

However, with the increase in the concentration of $H^+$ ion, it is known that more and more of the ion pair $HS_2O_3^-$ is formed, thereby leaving less and less of free $S_2O_3^{2-}$ for the above reversible reaction. So the effective concentration of the $S_2O_3^{2-}$ ion is not the same as its total concentration. Thus, the change of the ratio from 1:1 to 1:2 or 1:3 is explained without having to ascribe such formulae as $Fe(S_2O_3)_2^-$ and $Fe(S_2O_3)_3^{3-}$ for the labile coloured intermediate. However, in a subsequent communication, a direct and simple experimental evidence will be furnished to prove beyond doubt that the labile coloured complex possesses the formula $Fe^+S_2O_3$.

Haldar and Banerjee (*loc. cit.*) worked with aqueous solutions, while Page (*loc. cit.*) with acidic solutions. When our results obtained with aqueous as well as acidic solutions, as recorded in Tables II and III, are scrutinised, it can be seen that in case of aqueous solution without acid the % transmission at zero-time increases very considerably when the wave-length is changed from 500 mμ to 600 mμ. Further, it can be noticed that the difference between the transmissions at the two wave-lengths decreases with decrease in the volume of the solution of ferric salt and increases again, showing that the difference is dependent on both the solutions of the ferric salt and the thiosulphate. In case of acidic solutions of ferric salts, no such marked change in the %transmission with the change of wave-length occurred, and further difference between the two %transmissions was more or less the same. The minimum transmission or maximum absorption for aqueous solutions though occurs at 6:6 composition ratio of $Fe^{3+}:S_2O_3^{2-}$, yet the absorption of 7:5 ratio (higher $Fe^{3+}$) is very much nearer to it than that of the 5:7 ratio (higher $S_2O_3^{2-}$). The question now arises as to the reason for the marked change in the transmission in case of aqueous solution without acid due to the change in the wave-length. Formation of some complex would explain for such a change in absorption. But the formation of any new complex at zero-time to any considerable extent would not allow the composition ratio of 6:6 to afford the maximum absorption. So it leads to the inescapable conclusion that some complex is formed in very low concentration having high extinction coefficient.

In solutions of ferric salts the following equilibria are known to exist (Milburn and Vosburgh, *J. Amer. Chem. Soc.*, 1955, **77**, 1352),

$$Fe^{3+} + H_2O \rightleftharpoons FeOH^{2+} + H^+ \quad \ldots \quad \ldots \quad (2)$$

$$2FeOH^{2+} \rightleftharpoons Fe(OH)_2Fe^{4+} \quad \ldots \quad \ldots \quad (3)$$

reaction (3) preponderating at higher concentrations of $Fe^{3+}$ ion over reaction (2). It is evident that with the increase in concentration of $H^+$, the concentration of $FeOH^{2+}$ would correspondingly decrease, and consequently the concentration $Fe(OH)_2Fe^{4+}$. So in aqueous solution the concentration of this complex $Fe(OH)_2Fe^{4+}$ would be the maximum. Due to the association of two ferric ions, this complex would possess high extinction coefficient at lower wave-length region. In fact 60% of the differences is accounted for by the aqueous solution of the ferric salt at the required concentration. And since difference in absorption is also dependent on the volume of thiosulphate solution, it is under investigation whether the following reversible reaction

$$Fe(OH)_2Fe^{4+} + S_2O_3^{2-} \rightleftharpoons Fe(OH)_2Fe^{2+}(S_2O_3)$$

can also be taken into account along with reaction (3). The existence of these complexes would also explain the reason for no marked change in %transmission when acidic solutions were used. Also these would explain the dependence of the difference of absorption on the concentration of $Fe^{3+}$, $OH^-$ and $S_2O_3^{2-}$ ions. It has been noted from the experimental data recorded in Tables II and III that in case of aqueous solution

of ferric salts the absorption for the composition ratio 7 : 5 (higher $Fe^{3+}$) has almost the same absorption as the composition ratio 6 : 6, while that for the composition ratio 5 : 7 (higher $S_2O_3^{2-}$) is away from the absorption for the composition ratio 6 : 6. This is also explained as two $Fe^{3+}$ ions and either one or none of the $S_2O_3^{2-}$ ions are concerned in the formation of the complex or complexes responsible for stronger absorption in the shorter wave-length region of the visible range.

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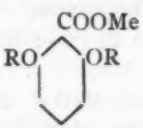
# STUDIES IN FRIES MIGRATION. PART XIII. FRIES REARRANGEMENT OF ACYL ESTERS OF METHYL 2:6-DIHYDROXYBENZOATE (METHYL γ-RESORCYLATE) AND 3:5-DIHYDROXYBENZOIC ACID (α-RESORCYLIC ACID)

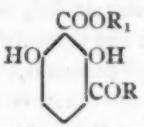
By J. I. Setalvad, G. C. Amin and N. M. Shah

The Fries rearrangement of methyl 2:6-diacetoxy- as well as -dibenzoyloxy-benzoate yields methyl 2:6-dihydroxy-3-acetyl- and -3 benzoyl-benzoates and the corresponding keto-acids respectively, hydrolysis occurring during the course of the migration. No diketone has been isolated. 3:5-Diacetoxy benzoic acid does not isomerise under the conditions of the Fries reaction.

In continuation of the previous work on the Fries migration of phenolic esters of hydroxybenzoic acids and their methyl esters (Shah *et al.*, this *Journal*, 1949, **26**, 235; 1952, **29**, 351), the Fries rearrangement of methyl 2:6-diacetoxy- and -dibenzoyloxy-benzoate (I: R = COMe and COPh) has been investigated.




(I)




(II)

Methyl 2:6-diacetoxybenzoate (I: R = COMe) isomerised to methyl 2:6-dihydroxy-3-acetylbenzoate (II: R = $R_1$ = Me) on heating with aluminium chloride at 110°. On alkaline hydrolysis of this ester, the corresponding acid (II: $R_1$ = H; R = Me) was obtained, which on decarboxylation afforded resacetophenone, thus establishing the structures of both the keto-acid and the ester. The migration at higher temperatures, 150°-155°, furnished only the keto-acid (II: R = Me; $R_1$ = H), hydrolysis occurring during the course of the migration. Similarly, methyl 2.6-dibenzoyloxybenzoate (I: R = COPh) afforded methyl 2:6-dihydroxy-3-benzoylbenzoate (II: R = Ph; $R_1$ = Me) and the corresponding acid (II: R = Ph; $R_1$ = H), when the reaction was carried out at room temperature in nitrobenzene. Without solvent, however, only the keto-acid was obtained, identical with the acid obtained by the hydrolysis of the keto-ester (II: R = Ph; $R_1$ = Me). Its constitution was proved by decarboxylating it to 4-benzoylresorcinol.

It is noteworthy that though both the favourable β-positions of the resorcinol nucleus are vacant for two migrating acyl groups to occupy, only one of the two β-positions is occupied by one of the two migrating acyl groups, leading to the formation of a mono-ketone. No 3:5-diketone derivative has been obtained, even if the migration is carried out at higher temperatures, which only favour the hydrolysis of the ester group. Thus, methyl 2.6-diacetoxybenzoate differs from its isomer, methyl 2:4 diacetoxybenzoate, which on migration yields methyl 3:5-diacetyl-2:4-dihydroxybenzoate, one of the acetyl groups occupying the difficultly accessible

γ-position, as described in a previous communication (Shah *et al.*, *loc. cit.*). The Friedel-Crafts acetylation of methyl 2:6-dihydroxybenzoate (I: R = H) yields similar results (cf. Desai, Radha and Shah, *Proc. Ind. Acad. Sci.*, 1945, **23A**, 305).

The Fries isomerisation of 3:5-diacetoxybenzoic acid (α-resorcylic acid) was attempted under various experimental conditions, but no ketonic product could be isolated, only the deacetylated acid having been recovered. The behaviour of this acid is analogous to that of *m*-hydroxybenzoic acid in this respect (Shah *et al.*, *loc. cit.* cf. Mauthner, *J. prakt. Chem.*, 1933, *ii*, **136**, 205). The Friedel-Crafts acetylation of methyl α-resorcylate as well as of the free acid was also found to be unsuccessful (cf. Mody and Shah, *Proc. Ind. Acad. Sci.*, 1951, **34A**, 77).

## EXPERIMENTAL

*Methyl 2:6-dihydroxybenzoate* (I: R = H) was prepared from 2:6-dihydroxybenzoic acid (Kelkar and Limaye, this *Journal*, 1935, **12**, 788) by Fischer-Spier's method and crystallised from dilute alcohol, m.p. 68-69°.

*Methyl 2:6-diacetoxybenzoate* (I: R = COMe) was prepared by sodium acetate-acetic anhydride method by heating the ester for 3 to 4 hours on a water-bath. It crystallised from dilute alcohol, m.p. 73-74°. It does not develop any colour with alcoholic ferric chloride and is insoluble in alkali. (Found: C, 57.0; H, 4.7. $C_{12}H_{12}O_6$ requires C, 57.1; H, 4.8 per cent).

*Fries Migration of* (I: R = COMe): *Formation of Methyl 2:6-Dihydroxy-3-acetylbenzoate* (II: R = $R_1$ = Me).—An intimate mixture of the ester (1 g.) and aluminium chloride (1.8 g.) was heated for an hour at 110°, HCl fumes evolving at about 65°. It was then treated with ice and HCl (5 c.c.). The solid obtained was collected and crystallised from dilute alcohol in small needles, m.p. 136-37°. (Found: C, 57.0; H, 4.8. $C_{10}H_{10}O_5$ requires C, 57.1; H, 4.8 per cent). The compound develops red colour with alcoholic ferric chloride and dissolves in alkali with yellow colour, but is insoluble in sodium bicarbonate. It is identical with the Friedel-Crafts acetylation product of Desai, Radha and Shah (*loc. cit.*).

The *semicarbazone*, prepared by the usual method, crystallised in needles from dilute acetic acid, m.p. 272-73° (decomp.). Desai, Radha and Shah report the same m. p.

*Hydrolysis*: *Formation of 2:6-Dihydroxy-3-acetylbenzoic Acid* (II: $R_1$ = H; R = Me).—The keto-ester (II: R = $R_1$ = Me) (0.5 g.) was dissolved in NaOH (10 c.c., 10%) and the yellowish solution was left overnight at room temperature. It was then acidified and the solution extracted with ether; the brownish solid obtained after removal of the ether crystallised from hot water in needles, m.p. 209-10° (decomp.). (Found: C, 54.9; H, 4.1. $C_9H_8O_5$ requires C, 55.1; H, 4.1 per cent). It develops a deep red colour with alcoholic ferric chloride.

Methyl 2:6-diacetoxybenzoate (I: R = COMe) was isomerised at 150°-155° as before; on working up the reaction mixture, no solid product was obtained. It was therefore extracted with ether from which a brownish product was obtained and identified as 2:6-dihydroxy-3-acetylbenzoic acid (II: $R_1$ = H; R = Me) after crystallisation from hot water by mixed melting point with the sample obtained above.

*Decarboxylation of the Acid* (II : $R_1$ = H ; R = Me).—The keto-acid (0.3 g.) was refluxed with water (20 c.c.) containing a few drops of HCl for 5 to 6 hours. On cooling, a solid separated which was collected and crystallised from hot water, m.p. 142-43°, identical with resacetophenone by mixed m.p. with an authentic sample.

*Methyl 2 : 6-dibenzoyloxybenzoate* (I : R = COPh) was prepared by Schotten-Baumann method by quickly adding benzoyl chloride (4.5 g., 2.2 *M*) to the ice-cold solution of methyl 2 : 6-dihydroxybenzoate (2.5 g., 1 *M*) in NaOH (15 c.c., 15%). It was well shaken and kept cool ; after half an hour, the smell of benzoyl chloride vanished completely. A pasty mass separated, which solidified on washing with cold water. It was then crystallised from dilute alcohol in yellow needles, m.p. 88°, yield 1.2 g. (Found : C, 70.5 ; H, 4.2. $C_{22}H_{16}O_6$ requires C, 70.2 ; H, 4.2 per cent). It is insoluble in alkali solution and does not respond to ferric chloride colour test.

*Fries Migration of* (I : R = COPh) : *Formation of Methyl 2 : 6-Dihydroxy-3-benzoylbenzoate* (II : $R_1$ = Me ; R = Ph) *and the corresponding Acid* (II : $R_1$ = H ; R = Ph).—(*a*). An intimate mixture of the benzoyloxy-ester (1.8 g., 1 *M*) and anhydrous aluminium chloride (2.5 g., 3.3 *M*) was heated at 120°-130° in an oil-bath for 1 hour. HCl gas was copiously evolved at 100°. The solid, obtained on working up the reaction mixture as before, dissolved completely with effervescence in dilute sodium bicarbonate solution (5%), which on acidification afforded a pale yellow solid, crystallising from dilute alcohol in pale yellow needles, m.p. 185° (efferv.), yield 0.8 g. (Found : Equiv., 256.8 ; C, 64.9 ; H, 3.8. $C_{14}H_{10}O_5$ requires equiv., 258 ; C, 65.1 ; H, 3.9 per cent).

(*b*). The above migration was repeated by keeping the reaction mixture of the benzoyloxy-ester (1.8 g.) and anhydrous aluminium chloride (2.5 g.), dissolved in dry nitrobenzene (15 c.c.), at room temperature for 24 hours, with $CaCl_2$ guard-tube. The reaction mixture was then worked up as usual. After removal of the nitrobenzene by steam-distillation, the residual solid was extracted with dilute sodium hydroxide solution (5%) and the yellow alkaline filtrate on acidification afforded a solid, which on treatment with sodium bicarbonate solution (5%) partly dissolved with effervescence. The bicarbonate-insoluble product crystallised from dilute alcohol in pale yellow shining needles, m.p. 121°. (Found : C, 66.2 ; H, 4.5. $C_{15}H_{12}O_5$ requires C, 66.1 ; H, 4.4 per cent). The solid obtained on acidifying the bicarbonate filtrate was identified as the keto-acid (II : $R_1$ = H ; R = Ph). Both the keto-ester and acid dissolve in alkali solution with a yellow colour and develop a red colour with alcoholic ferric chloride.

The *keto-ester* (II : $R_1$ = Me ; R = Ph), obtained above, was hydrolysed to the corresponding acid (II : $R_1$ = H ; R = Ph) by sodium hydroxide (10%), the acid being identified as 2 : 6-dihydroxy-3-benzoylbenzoic acid by mixed m.p.

*Decarboxyiation of the keto-acid* (II : $R_1$ = H ; R = Ph) (0.5 g.), by heating it with water (30 c.c.), acetic acid (2 c.c.) and a few drops of HCl (conc.) for 15-18 hours on a sand-bath furnished 4-benzoylresorcinol, m.p. and mixed m.p. with an authentic sample, 144-45°.

3 : 5-*Dihydroxybenzoic acid* or α-resorcylic *acid* ("Org. Syn"., 1941, vol. 21, p. 27), acetylated by acetic anhydride-pyridine method, afforded 3 : 5-diacetoxybenzoic acid, which was crystallised from dilute acetic acid in colorless prismatic needles, m.p. 156-57°.

The Fries migration of the above diacetoxy compound was attempted under a variety of conditions but only the deacetylated product was recovered.

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# ELECTROCHEMICAL ASPECTS OF RUBBER LATEX, COMPOUNDING INGREDIENTS AND THEIR MIXTURES

By Brojeswar Roy

The electrophoretic speeds of rubber latex particles as well as of the colloidally dispersed compounding agents, e. g., zinc oxide, sulphur, China clay and of mixtures of them as are generally employed in baths for electrodeposition, have been measured in a microcataphoresis cell of the Northrup-Kunitz type. ζ-Potential of latex, calculated from the electrophoretic speeds, is considerably influenced by the presence of the compounding agents. Sulphur sol having the lowest ζ-potential tends to reduce that of latex as well as of others in a mixture, whereas zinc oxide and China clay, which have higher ζ potentials, tend to increase the value for latex. A mixture of zinc oxide and China clay shows the highest (60 mV) ζ-potential compared to latex itself (ζ = 28.8 mV). The isoelectric point of rubber latex was measured by calculating ζ-potential from electrophoretic speed at different $p_H$ values, which were maintained by means of $Na_2CO_3$-$NaHCO_3$ buffers at the higher range and acetate buffers at the lower range. The I. P. was 4.2 and was lowered in presence of zinc oxide and China clay sols to 3.0 and 3.8 respectively, but increased to 5.1 in presence of sulphur sol.

The negative charge of rubber particles in Hevea latex has been demonstrated by a number of workers (Henry, *compt. rend.*, 1907, **144**, 431 ; Sheppard, *Trans. Amer. Electrochem. Soc.*, 1937, **52**, 47 ; Kemp and Twiss, *Trans. Faraday Soc.*, 1936, **32**, 890 ; Blow, *J. Soc. Chem. Ind.*, 1938, **57**, 116T) and has been taken advantage of in the electrodeposition of rubber from latex bath. The ingredients of latex bath are quite numerous and include substances like sulphur, zinc oxide, China clay, wetting agents etc., each having a more or less specific role to play. The influence of these ingredients on the mobilities of rubber particles has not been properly assessed, although it is known that electrodeposition is affected by the added substances to an appreciable extent. The influence of small amounts of electrolytes (Sheppard, *loc. cit.*) and wetting agents (Blow, *loc. cit.*) on the electrophoretic mobility of rubber particles was noted by several workers.

In the present investigation, some of the ingredients of the bath, e. g., China clay, sulphur and zinc oxide, were obtained in the colloidal form and the electrophoretic mobility was measured individually and in mixtures of them, both amongst themselves and with latex. One of the objects of this study was to assess, if possible, the individual effect of the ingredients or mixtures of them on the electrophoretic mobility or ζ-potential and subsequently on electrodeposition of latex. The electrodeposition of rubber finds, as is well known, industrial applications in the preparation of microporous rubber (Flint, "The Chemistry and Technology of Rubber Latex", pp. 441, 561).

## Experimental

Hevea latex, used in the present study, was obtained in a concentrated form from South India. The colloid content was determined by evaporating and drying a known volume of the suspension at 75° ± 2°. The latex was suitably diluted for subsequent experiments.

China clay was boiled with dilute HCl, washed free of acid and then peptised with ammonium hydroxide. The sol was diluted to 3%. 1% Zinc oxide sol was prepared

by mixing 1 part zinc oxide, 1 part water and 1% ammonium caesinate on dry weight of zinc oxide and then by requisite dilution. Colloidal sulphur (0.02%) was prepared by pouring a saturated solution of sulphur in absolute alcohol to a large volume of distilled water, and then removing as much alcohol as possible.

*Measurement of Cataphoretic Speed and Electrokinetic Potential*

The speed of the colloidal particles was measured at 0.79 depth in a microcataphoresis cell, designed by Abramson in modification of Northrup-Kunitz type. The $\zeta$-potential was calculated by the simple Helmholtz relation :

$$\zeta = \frac{4\pi\eta\, cAd\,(300)^2}{D\, i\, R\, t}$$

where, $\eta$ = viscosity of the medium (0.01 poise approximately) ; $D$ = dielectric constant of the medium (80) ; $A$ = cross-section of the electrophoretic cell (0.073 $cm^2$.) ; $i$ = current in amperes ; $c$ = cell constant (0.224) ; $d$ = length of 50 divisions of the scale of the microscope (0.0417 cm.) ; $t$ = time in seconds required to move 50 divisions ; $R$ = resistance in ohms of the sol measured in the conductivity cell.

The calculated $\zeta$-potentials of sols, diluted 10 to 100 times, are shown in Table I. The $p_H$ was measured with the help of a glass electrode.

TABLE I

| Colloidal sol. | Conc. | $p_H$. | $\zeta$-Potential. |
|---|---|---|---|
| Latex | 0.034 % | 8.1 | 28.8 mV |
| China clay | 0.5 | 8.8 | 37.7 |
| Zinc oxide | 0.1 | 8.2 | 33.6 |
| Sulphur | 0.02 | 7.2 | 25.8 |

The dilute sols were mixed together in equal volumes and $\zeta$-potentials were measured as usual. In mixtures containing latex as one of the ingredients, the particles which were singled out for velocity measurement were invariably those of latex. In the first four mixtures, recorded in Table II, the China clay particles could be easily spotted for measurement.

TABLE II

| Mixtures of sols. | $p_H$. | $\zeta$-Potential (mV). |
|---|---|---|
| China clay + sulphur | 8.2 | 27.3 (China clay) |
| Zinc oxide + sulphur | 7.3 | 29.5 " |
| China clay + sulphur + zinc oxide | 7.6 | 24.3 " |
| China clay + zinc oxide | 8.0 | 36.3 " |
| Latex + China clay | 7.1 | 48.3 (latex) |
| Latex + zinc oxide | 7.6 | 49.8 " |
| Latex + China clay + zinc oxide | 7.4 | 60.1 " |
| Latex + sulphur | 7.8 | 28.1 " |
| Latex + sulphur + zinc oxide | 7.9 | 29.7 " |
| Latex + sulphur + China clay | 8.4 | 32.3 " |
| Latex + sulphur + China clay + zinc oxide | 8.3 | 35.6 " |

*Isoelectric $p_H$ of Latex*

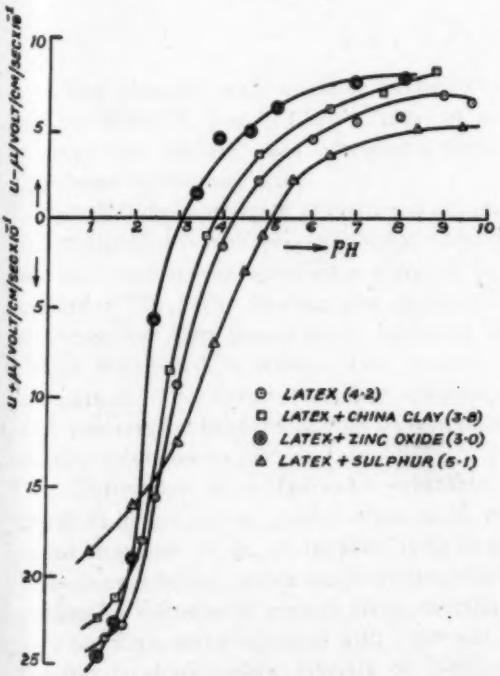
The variation of the cataphoretic speed of the rubber particles with $p_H$, individually and in mixture with some of the colloidal compounding ingredients, was measured by using various buffer solutions ranging from $p_H$ 1 to 10. Sodium carbonate-bicarbonate buffers were employed in the alkaline range, while the acetate buffers were used in the acid range. For lower $p_H$, a hydrochloric acid solution was used. With the exception of the concentrated solutions having very low $p_H$, the ionic concentration was maintained at 0.01 *N*. The results are graphically shown in Fig. 1, from which the isoelectric $p_H$ is found out to be 4.2.

## DISCUSSION

The ζ-potential of latex and the compounding ingredients appears to decrease in the order: China clay, zinc oxide, latex, sulphur, which is also the decreasing order of the $p_H$ of the suspensions.

An examination of the graph in Fig. 1 reveals that sulphur sol, which has the lowest potential amongst the compounding ingredients, lowers the ζ-potential of the others by its presence. A mixture of zinc oxide and China clay sol has an intermediate potential, but in presence of sulphur the potential is considerably lowered. In presence of both China clay as well as zinc oxide, the ζ-potential of latex increases and reaches the highest value when the three are present together. But the addition of sulphur to all these mixtures markedly lowers the ζ-potential. The ζ-potential of sulphur is of course increased in presence of others.

FIG. 1




The isoelectric point of latex is found to be 4.2, which is somewhat higher than the value (3.7) observed by Subramanyan, Ghosh and Rao (*J. Sci. Ind. Res.*, 1948, **7B**, 77). This is lowered to $p_H$ 3.0 and 3.8 respectively in presence of zinc oxide and China clay sols but increased to 5.1 in presence of sulphur sol which destabilises the latex, as has been shown by the potential measurements. The effect of zinc oxide and China clay sols is to stabilise the latex towards acids. Zinc oxide lowers the isoelectric point to a greater extent than China clay. This is perhaps due to the presence in zinc oxide

of ammonium caesinate, which is a greater stabilising agent than ammonia present in China clay. The destabilising effect of sulphur sol may be partly due to the presence of a little alcohol that could not be removed from it.

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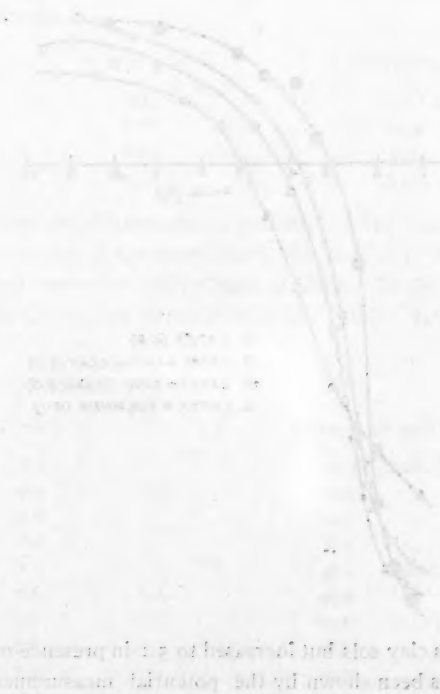

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# ANALYTICAL ASPECTS OF SOME ORGANIC COMPOUNDS. PART III. 1-HYDROXY-2-NAPHTHOIC ACIDS IN THE ESTIMATION OF ZIRCONIUM

By Sachindra Kumar Datta

Analytical behaviour of 1-hydroxy-2-naphthoic acid and its nitro and bromo derivatives was studied. Thorium and zirconium furnished quantitative precipitation with these acids. Gravimetric estimation of zirconium by these reagents and its separation from other elements have been described.

In a previous communication (this *Journal*, 1955, **32**, 687), several phenolic acids were studied for their analytical applications, and most of them were found suitable in the determination of thorium and zirconium. In the present paper, the analytical behaviour of another phenolic acid, namely 1-hydroxy-2-naphthoic acid, and its derivatives, like 1-hydroxy-4-bromo-2-naphthoic and 1-hydroxy-4-nitro-2-naphthoic acids, has been examined. The preparation of these acids and their use in the quantitative estimation of zirconium and its separation from other elements are also described herein.

## Experimental

The phenolic acids could be prepared by heating the sodium phenate in a stream of $CO_2$ (Kolbe, *J. prakt. Chem.*, 1874, **10**, 94). The method of Desai and Sethna (this *Journal*, 1951, **28**, 215) was modified a little in the present work for the preparation of 1-hydroxy-2-naphthoic acid.

Potassium bicarbonate (2 mol.) was dissolved in the minimum quantity of hot water and α-naphthol (1 mol.) was gradually added to it with stirring to make a thick paste, and the mixture was heated in a glycerine bath, maintained at 120°-130°, in a slow current of $CO_2$. The heating was continued for 5 to 6 hours till the melt solidified. The mass was then poured into a beaker of water and stirred well till the solid was evenly distributed in water. The mixture was filtered to remove the unchanged α-naphthol. The filtrate was then acidified with HCl (dil.). 1-Hydroxy-2-naphthoic acid separated in the form of a pink precipitate which was crystallised from spirit till its melting point rose to 196°.

*Derivatives of 1-Hydroxy-2-naphthoic Acid.*—The naphthoic acid (18.8 g.), dissolved in 100 c.c. of glacial acetic acid, was cooled in ice and to this mixture was added dropwise 18 g. of bromine (5%) in glacial acetic acid solution. The resulting blue-violet solution, which was kept under ice for 15 minutes, on pouring into water, produced a voluminous greyish violet precipitate. The precipitate was filtered, washed with hot water and then boiled with water and the mixture was filtered hot. On cooling the filtrate shiny yellow crystals of 1-hydroxy-4-bromo-2-naphthoic acid separated, which was recrystallised from water, m.p. 130°. The residue (that remained on boiling with water) was discarded. It was a monobromo derivative. (Found : Br, 29.87. Calc. for $C_{11}H_7O_3Br$ : Br, 29.96 per cent).

Fuming nitric acid (15 g.) was slowly added in small portions to a solution of the hydroxynaphthoic acid (15 g.) in alcohol (80%), well cooled in ice. The red-violet liquid was allowed to stand for 10 minutes in ice and then poured into a large volume of water; an ash-coloured precipitate appeared at once. This was filtered and washed with water till it was free of nitric acid. The precipitate was then boiled with water and the mixture was filtered hot. On cooling the filtrate, a yellow coloured solid came out. This was recrystallised from hot water, m.p. 165°, (decomp. 200°) and was identified to be the 1-hydroxy-4-nitro-2-naphthoic acid. (Found : N, 5.87. Calc. for $C_{11}H_7O_5N$ : N, 6.0 per cent).

*Possible Use in Qualitative and Quantitative Analysis*

It was observed that 1-hydroxy-2-naphthoic acid developed a bluish violet coloration with copper and blue coloration with $Fe^{III}$ at $p_H$ 3.09. The nitro and bromo compounds produced bluish green colour with copper and yellow-green and bluish green respectively, with iron at the same $p_H$. All the three acids developed orange coloration with uranium at $p_H$ 3.61. They produced white or yellowish white precipitates with $Hg^I$, the precipitation, however, was not complete. Quantitative precipitations of thorium and zirconium were obtained with all these acids at $p_H$ 4.58. The thorium salts of 1-hydroxy-2-naphthoic acid and its nitro and bromo derivatives are white, yellowish white and orange, respectively, while those of zirconium are white, yellow and brown.

*Gravimetric Determination of Zirconium*

A standard stock solution of zirconium nitrate (Merck's reagent quality) was prepared and the zirconium content was determined with benzilic acid (Venkatramaniah and Rao, this *Journal*, 1951, **28**, 257 ; Klingenberg and Mendel, *Anal. Chem.*, 1954, **26**, 754). Stock solutions of various metal salts (all reagent grade), like iron (ic), barium, calcium, strontium, cobalt, nickel, chromium, manganese, titanium and uranium were also prepared and their metal content was estimated by standard methods.

1-Hydroxy-2-naphthoic acid is sparingly soluble in water. Its 0.5% solution was prepared in boiling water. Cold aqueous solution (2%) of its sodium salt was also used. The bromo and nitro derivatives were more soluble in hot water than the parent substance, so their 1% solutions in hot water were used for estimations.

*Procedure.*—Aliquot quantities of zirconium solutions, rendered neutral to Congo red, were heated nearly to boiling and then the reagent solutions in slight excess were added. No immediate precipitate appeared, but on adding about 1 c.c. of 2% ammonium acetate solution precipitation occurred at once. 1-Hydroxy-2-naphthoic acid afforded a white flocculent precipitate ; the nitro and bromo derivatives produced yellow and brown gelatinous precipitates respectively. On heating the beaker for a few minutes the precipitates collected in the form of easily filtrable mass towards the centre of the beaker. In no case the precipitates should be boiled. The nature of the precipitates obtained with the sodium salt of hydroxynaphthoic acid was much better than that with the free acid. The precipitate was filtered and washed with hot water several times and then ignited to zirconia to a constant weight. The results are shown in Table I.

TABLE I

*Estimation of zirconium with hydroxynaphthoic acid.*

| No. | $ZrO_2$ taken. | $ZrO_2$ found by 1-OH-N. | $ZrO_2$ found by Na-salt of 1-OH-N. | $ZrO_2$ found by 1-OH-4-$NO_2$-N. | $ZrO_2$ found by 1-OH-4-Br-N. |
|---|---|---|---|---|---|
| 1 | 0.0462 g. | 0.0461 g. | 0.0462 g. | 0.0461 g. | 0.0464 g. |
| 2 | 0.0308 | 0.0308 | 0.0310 | 0.0308 | 0.0310 |
| 3 | 0.0231 | 0.0229 | 0.0231 | 0.0233 | 0.0233 |
| 4 | 0.0154 | 0.0150 | 0.0151 | 0.0152 | 0.0153 |
| 5 | 0.0077 | 0.0072 | 0.0074 | 0.0075 | 0.0077 |
| 6 | 0.0039 | 0.0023 | 0.0028 | 0.0030 | 0.0032 |
| 7 | 0.0019 | ... | ... | 0.0015 | 0.0017 |

N stands for naphthoic acid.

From the above table it is evident that the bromo derivative is the most effective precipitating agent of all the reagents, then come nitro compound, sodium salt of hydroxynaphthoic acid and the free hydroxynaphthoic acid in the decreasing order of effectiveness.

*Effect of Hydrogen-ion Concentration on the Precipitation of Zirconium.*—With a view to ascertaining a suitable $p_H$ range for each of the reagents, for the quantitative precipitation of zirconium, estimations were carried out in a similar manner in solutions having different $p_H$ values. The results are shown in Table II.

TABLE II

$ZrO_2$ taken = 0.0154 g.

| $ZrO_2$ found (g.) by | $p_H$ of the final solutions. 3. | 3.6. | 4.2. | 4.8. | 5.0 | 5.5 |
|---|---|---|---|---|---|---|
| 1-OH-N | 0.0120 | 0.0144 | 0.0152 | 0.0153 | 0.0152 | 0.0153 |
| 1-OH-N (Na salt) | 0.0129 | 0.0147 | 0.0153 | 0.0154 | 0.0155 | 0.0156 |
| 1-OH-4-$NO_2$-N | 0.0130 | 0.0150 | 0.0152 | 0.0154 | 0.0155 | 0.0156 |
| 1-OH-4-Br-N | 0.0141 | 0.0152 | 0.0154 | 0.0155 | 0.0155 | 0.0156 |

It is seen from the $p_H$ studies that the bromo derivative has a very wide range of $p_H$, *i. e.* from 3.6 to 5.5, for the quantitative precipitation of zirconium, while the range for the others is too narrow.

*Separation of Zirconium from Foreign Ions*

In order to study the interference by other metals, different quantities of metal solutions were added to a definite amount of zirconium nitrate solution and the precipitation of zirconium was carried out as before. Separation from most of the metals, like Ca, Ba, Sr, Al, Zn, Mg, Ti etc., may be carried out with these reagents by single precipitation, whereas iron, cobalt, nickel and uranium require double precipitation. As thorium is co-precipitated by all these reagents, within the same range as zirconium, it is not possible to separate Zr from Th. The results are recorded in Table III.

TABLE III

| $ZrO_2$ taken. | Metal oxide added. | $ZrO_2$ found by 1-OH-N (Na-salt). | $ZrO_2$ found by 1-OH-4-$NO_2$-N. | $ZrO_2$ found by 1-OH-4-Br N. |
|---|---|---|---|---|
| 0.0231 g. | CaO 0.0316 g. | 0.0230 g. | 0 0230 g. | 0.0231 g. |
| ,, | BaO 0.0250 | 0.0231 | 0.0232 | 0.0232 |
| ,, | SrO 0.0218 | 0.0230 | 0.0231 | 0.0233 |
| ,, | * $Fe_2O_3$ 0.0181 | 0.0238 | 0.0236 | 0.0235 |
| ,, | $Al_2O_3$ 0.0232 | 0.0233 | 0 0 32 | 0.0232 |
| 0.0154 | ZnO 0.0153 | 0.0151 | 0.0152 | 0 0153 |
| ,, | MgO 0 0190 | 0.0152 | 0.0152 | 0.0153 |
| ,, | $TiO_2$ 0.0252 | 0 0151 | 0.0152 | 0 0154 |
| ,, | * CoO 0.0167 | 0.0156 | 0.0156 | 0.0155 |
| ,, | * NiO 0.0266 | 0.0157 | 0.0156 | 0.0155 |
| ,, | * $U_3O_8$ 0.0157 | 0.0156 | 0.0155 | 0.0155 |

* Indicate double pptn.

*Composition of Zirconium Salts*

The zirconium salts of all the acids were prepared as before ; they were washed with boiling water several times and then with hot alcohol in order to free them from organic acids, and finally with ether. They were dried in an electric oven at 110°-115° to constant weights. Aliquot amounts of these salts were ignited in a platinum crucible and weighed as $ZrO_2$. The results of analysis show that zirconium cannot be estimated by direct weighing with any of these acids, as the salts are all basic in nature. One atom of zirconium is associated with three hydroxyl groups and one acid residue in a molecule of all the salts.

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# STUDIES ON THE FORMATION OF ALUMINATES

By Sarju Prasad and Jai Beni Prasad Tripathi

Aluminates of Ba, Sr, Mg, Zn and Ni have been prepared at high temperatures.

Tanaka (*Bull. Chem. Soc., Japan*, 1941, **16**, 186) obtained $MgO.Al_2O_3$ by heating a mixture of MgO and $Al_2O_3$ at 1400° for 10 hours. Noguchi (*J. Japan Ceram. Assoc.*, 1948, **56**, 77) also obtained the same compound by heating equimolar mixtures of MgO and $Al_2O_3$ at 1900°, using a large number of mineralisers.

Mourelo (*Rev. gen. Sci.*, 1915, **26**, 399) synthesised zinc aluminate by heating a mixture of zinc oxide, alumina and boric oxide. Meunier (*Bull. Soc. Min.*, 1887, **10**, 194) prepared zinc aluminate by heating to bright redness a mixture of alumina, zinc oxide, cryolite and aluminium chloride, and Deville and Caron (*Compt. rend.*, 1858, **46**, 764) by vaporising a mixture of zinc and aluminium fluorides in the presence of boric oxide.

Jander and Grob (*Z. anorg. Chem.*, 1940, **245**, 67), Milligan and Merten (*J. Phys. Chem.*, 1946, **50**, 465) and Chaumeton, Bilde and Devaux (*Compt. rend.*, 1951, **232**, 1556) prepared $NiO.Al_2O_3$ by heating the mixtures of corresponding oxides at various temperatures.

The present investigation was undertaken with a view to studying the formation of barium aluminates by the interaction of bauxite and barytes, $Ba(OH)_2$ or $Ba(NO_3)_2$, and strontium aluminates by the interaction of bauxite and strontium sulphate or strontium nitrate at high temperatures. The work was further extended to the preparation of aluminates of Mg, Zn and Ni.

## Experimental

All the chemicals used were of Merck E.P. quality. The bauxite used was from Katni and on analysis furnished the composition recorded in Table I.

Table I

| | Expt. No. 1. | Expt. No. 2. | Average. |
|---|---|---|---|
| Moisture | 1.51% | 1.52% | 1.515% |
| Combined water | 28.54 | 28.52 | 28.53 |
| $SiO_2$ | 7.28 | 7.26 | 7.27 |
| ($TiO_2$ & $Fe_2O_3$) | 8.00 | 8.06 | 8.03 |
| $Al_2O_3$ | 54.40 | 54.30 | 54.35 |

Several mixtures were prepared by weighing out accurately bauxite and other constituents in different proportions. They were mixed in an agate pestle and mortar, transferred into the crucibles and heated at high temperatures for different intervals of time. Duplicate experiments were carried out in each case. The heated mass was taken out, powdered well and weighed. A known weight of this mixture

was taken for analysis. It was first extracted with water and afterwards with dilute HCl, and the quantities of $Al_2O_3$, BaO or SrO were estimated in both the extracts separately. In experiments where bauxite was heated with $Ba(NO_3)_2$, $Ba(OH)_2$ or $Sr(NO_3)_2$, the water extract was not analysed.

TABLE IIA

*Barium aluminate.*

Time of heating = 5 hours.

| Expt. No. | Bauxite : barytes | Temp. | Water-soluble (BaO). | Dil.HCl-soluble. BaO. | Dil.HCl-soluble. $Al_2O_3$ | Compound. |
|---|---|---|---|---|---|---|
| 1 | 10 : 15 | 1400°-1450° | ... | 6.524 g. | 1.452 g. | 3 $BaO.Al_2O_3$ |
| 2 | 10 : 15 | ,, | ... | 6 551 | 1.460 | 3 $BaO.Al_2O_3$ |
| 3 | 10 : 50 | ,, | 7.128 g | 14 528 | 4 806 | 2 $BaO.Al_2O_3$ |
| 4 | 10 : 50 | ,, | 7.166 | 14 523 | 4.802 | ,, |
| 5 | 10 : 25 | 1200°-1250° | 5.653 | 11.464 | 3.812 | ,, |
| 6 | 10 : 25 | ,, | 5 682 | 11 448 | 3 803 | ,, |
| 7 | 10 : 40 | ,, | 3.624 | 7.286 | 2.422 | ,, |
| 8 | 10 : 40 | ,, | 3.628 | 7.274 | 2.421 | ,, |

TABLE IIB

Time of heating = 2.5 hours. Temp. = 1000°-1050°.

| Expt. No. | Bauxite. | $Ba(NO_3)_2$. | Dil.HCl-soluble. BaO. | Dil.HCl-soluble. $Al_2O_3$. | Compound. |
|---|---|---|---|---|---|
| 9 | 1.0 g. | 3.5 g. | 0 530 g. | 0.176 g. | 2 $BaO.Al_2O_3$ |
| 10 | 1.0 | 3.5 $Ba(OH)_2.8H_2O$. | 0.532 | 0.177 | ,, |
| 11 | 1.0 | 4.0 | 1.180 | 0.393 | 2 $BaO.Al_2O_3$ |
| 12 | 1.0 | 4.0 | 1.178 | 0.392 | ,, |

TABLE IIIA

*Strontium aluminate.*

Time of heating = 5 hours.

| Expt. No. | Bauxite : $Sr(SO_4)$. | Temp. | Water-soluble. | Dil.HCl-soluble. SrO. | Dil.HCl-soluble. $Al_2O_3$. | Compound. |
|---|---|---|---|---|---|---|
| 13 | 10 : 15 | 1400°-1450° | ... | 5 080 g. | 2.520 g. | 2 $SrO.Al_2O_3$ |
| 14 | 10 : 15 | ,, | ... | 5.110 | 2.522 | ,, |
| 15 | 10 : 20 | ,, | ... | 5.478 | 2.710 | ,, |
| 16 | 10 : 20 | ,, | ... | 5.480 | 2.712 | ,, |
| 17 | 10 : 25 | ,, | ... | 8 362 | 4.120 | ,, |
| 18 | 10 : 25 | ,, | ... | 8 358 | 4.118 | ,, |
| 19 | 10 : 40 | ,, | ... | 9.124 | 4.556 | ,, |
| 20 | 10 : 40 | ,, | ... | 9 126 | 4.554 | ,, |
| 21 | 10 : 15 | 1200°-1250° | 2.464 g. | 4.028 | 1.326 | 3 $SrO.Al_2O_3$ |
| 22 | 10 : 15 | ,, | 2.628 | 4.034 | 1.328 | ,, |
| 23 | 10 : 40 | ,, | 3.240 | 6 920 | 2.268 | ,, |
| 24 | 10 : 40 | ,, | 3.260 | 6.925 | 2.274 | ,, |

TABLE IIIB

Time of heating = 5 hours. Temp. = 1000°-1050°.

| Expt. No. | Bauxite : $Sr(NO_3)_2,4H_2O$. | Dil. HCl-soluble. SrO. | $Al_2O_3$. | Compound. |
|---|---|---|---|---|
| 25 | 10 : 40 | 5.140 g. | 1.680 g. | 3 $SrO.Al_2O_3$ |
| 26 | 10 : 40 | 5.142 | 1.681 | ,, |

*Magnesium and Zinc Aluminates.*—Basic aluminium acetate and $MgSO_4$, $7H_2O$, $MgCl_2,6H_2O$ or $ZnSO_4,7H_2O$ were weighed, mixed in an agate pestle and mortar, transferred into the crucibles and heated at high temperatures. The heated mass was taken out, boiled with dilute HCl and filtered. The residue was washed with hot water, dried and weighed. A known weight of the residue was taken for analysis. It was fused with $Na_2CO_3$, $KNO_3$ and a small quantity of NaOH and the fused mass extracted with dilute HCl. It was filtered and washed with hot water. The residue was fused again with a mixture of $Na_2CO_3$, $KNO_3$ and NaOH, and the process repeated till the whole mass was extracted with dilute HCl. The quantities of $Al_2O_3$ and MgO or ZnO were estimated in both the extracts separately.

TABLE IV

*Magnesium aluminate.*

Time of heating = 2 hours. Basic Al acetate taken = 3.0 g.

| Expt. No. | $MgSO_4,7H_2O$. | Temp. | Dil.HCl-soluble (MgO). | Analysis of the residue. MgO. | $Al_2O_3$. | Compound. |
|---|---|---|---|---|---|---|
| 27 | 3.06 g. | 1400° | Negligible | 0.4980 g. | 1.2740 g. | $MgO.Al_2O_3$ |
| 28 | 3.06 | ,, | ,, | 0 4978 | 1.2746 | ,, |
| 29 | 6.12 | ,, | 0.5070 g. | 0.4920 | 1.2702 | ,, |
| 30 | 6.12 | ,, | 0.5072 | 0.4914 | 1.2700 | ,, |
| | $MgCl_2,6H_2O$. | | | | | |
| 31 | 2.6 | 1200° | Negligible | 0.4976 | 1.2724 | ,, |
| 32 | 2.6 | ,, | ,, | 0.4964 | 1.2730 | ,, |
| 33 | 5 2 | ,, | 0.4984 g. | 0.5002 | 1 2746 | ,, |
| 34 | 5.2 | ,, | 0.4992 | 0.4996 | 1.2742 | ,, |

TABLE V

*Zinc aluminate.*

Time of heating = 2 hours. Temp. = 1200°. Basic Al acetate taken = 2.95 g.

| Expt. No. | $ZnSO_4,7H_2O$. | Dil.HCl-soluble (ZnO). | Analysis of the residue. ZnO. | $Al_2O_3$. | Compound. |
|---|---|---|---|---|---|
| 35 | 3.53 g. | ... | 0.992 g. | 1.240 g. | $ZnO.Al_2O_3$ |
| 36 | 3.53 | ... | 0.988 | 1.241 | ,, |
| 37 | 7.06 | 0.995 g. | 1.002 | 1.251 | ,, |
| 38 | 7 06 | 1.002 | 1.001 | 1.253 | ,, |

*Nickel Aluminate.*—Basic aluminium acetate and nickel acetate (hydrated) were weighed accurately, mixed in an agate pestle and mortar and transferred into the crucible and heated at high temperatures. The heated mass was taken out and extracted with dilute HCl (2-3*N*). The residue was boiled with HCl (10 *N*), filtered, washed with hot water and analysed. The quantities of $Al_2O_3$ and NiO were estimated in HCl (conc.) extract.

TABLE VI

*Nickel aluminate.*

Time of heating = 2 hours. Temp. = 1200°. Basic Al acetate taken = 3.30 g.

| Expt. No. | Ni-acetate. | Dil. HCl-soluble (NiO). | Conc. HCl-soluble. NiO. | Conc. HCl-soluble. $Al_2O_3$. | Compound. |
|---|---|---|---|---|---|
| 39 | 2.37 g. | 0.408 g. | 0.316 g. | 0.430 g. | $NiO.Al_2O_3$ |
| 40 | 2.37 | 0.412 | 0 311 | 0.426 | ,, |
| 41 | 4.74 | 1.042 | 0 413 | 0.562 | ,, |
| 42 | 4.74 | 1.046 | 0.411 | 0.563 | ,, |

DISCUSSION

*Barium Aluminate.*—It is evident from Table IIA that in all the cases the water extract provides sufficient quantities of BaO, but not even traces of $Al(OH)_3$, and the acid extract affords BaO as well as $Al(OH)_3$. As alumina present in the bauxite was heated at very high temperatures, which would render it insoluble in dilute or concentrated HCl, barium aluminates must have been formed from which alumina had been extracted by dilute acid.

In expts. No. 1 and 2, where the mixture containing bauxite and barytes in the ratio of 10:15 was heated at 1400°-1450°, the mass was glass-like and could be taken out of the crucible with difficulty. When boiled with water for a long time it did not disintegrate and the water extract neither responded to the test for $Ba(OH)_2$, nor for $Al(OH)_3$; when boiled with dilute HCl it, however, disintegrated. The ratio of BaO to $Al_2O_3$ obtained in the acid extract indicates the compound as $3BaO.Al_2O_3$. When the above experiments were repeated with larger quantities of barytes, the mass was not so hard and it disintegrated when boiled with water; the water extract provided sufficient quantities of BaO but no $Al_2O_3$. The ratio of BaO to $Al_2O_3$ in the acid extract indicates the composition of the compound as $2BaO.Al_2O_3$. If BaO in the water extracts be added to BaO in the acid extract, the compound formed would correspond to $3BaO.Al_2O_3$.

Expts. No. 5 and 6 show that only $Ba(OH)_2$ is obtained in the water extracts and both $Ba(OH)_2$ and $Al(OH)_3$ in the acid extracts. From the ratio of BaO to $Al_2O_3$ in the acid extract the compound appears to be $2BaO.Al_2O_3$. If BaO from the water and the acid extracts be added together, the ratio BaO to $Al_2O_3$ will correspond to $3BaO.Al_2O_3$.

The above experiments show that the compound formed is $3BaO.Al_2O_3$ in all the cases which, when treated with water, hydrolyses to $Ba(OH)_2$ and $2BaO.Al_2O_3$; when bauxite is heated with $Ba(OH)_2$ or $Ba(NO_3)_2$, the ratio of BaO to $Al_2O_3$ in the acid extract indicates the compound as $2BaO.Al_2O_3$.

*Strontium Aluminates.*—Expts. No. 13-20, carried out at 1400°-1450°, show that $Sr(OH)_2$ is not obtained in the water extract. The ratio of SrO to $Al_2O_3$ in the acid extract shows the compound to be $2SrO.Al_2O_3$.

Expts. No. 21-24, carried out at 1200°-1250°, afforded enough quantities of $Sr(OH)_2$ with water. From the ratio of SrO to $Al_2O_3$ in the acid extract the compound appears to be $3SrO.Al_2O_3$. Bauxite when heated with $Sr(NO_3)_2$ at 1000°-1050° furnished the same compound, $3SrO.Al_2O_3$.

From these results it is definitely established that $3SrO.Al_2O_3$ is formed at 1200°-1250° and $2SrO.Al_2O_3$ at 1400°-1450°.

*Magnesium Aluminate.*—From Table IV it is evident that the compound formed by heating a mixture of $MgSO_4$, $7H_2O$ and basic aluminium acetate at 1400° is $MgO.Al_2O_3$, which is insoluble in water and in HCl (dil. and conc.). Expts. No. 29 and 30 were carried out with double the quantity of magnesium sulphate but the same compound was formed and unchanged MgO was extracted with HCl. The experiment, when repeated with $MgCl_2,6H_2O$ mixed with NaCl instead of $MgSO_4$, $7H_2O$, furnished the same compound. In this case the reaction occurred at a much lower temperature and was over in a shorter duration.

*Zinc Aluminate.*—In expts. No. 35 and 36, when the molar ratio of $ZnSO_4,7H_2O$ and aluminium acetate was 1:1, neither $Al_2O_3$ nor ZnO was obtained in the acid extract. Expts. No. 37 and 38 were carried out with double the quantity of $ZnSO_4$, $7H_2O$ and the fused mass, when extracted with HCl (conc.) gave a large amount of ZnO, but no $Al_2O_3$. The residue on analysis furnished the compound, $ZnO.Al_2O_3$.

*Nickel Aluminate.*—Expts. No. 39 and 40 show that the only aluminate which is formed by heating calculated quantities of nickel and aluminium acetates corresponding to mono- and di-nickel aluminates at about 1200° is $NiO.Al_2O_3$. When double the quantity of nickel acetate is used, no dinickel aluminate is formed. The unchanged NiO was extraced with dilute HCl, and the ignited $Al_2O_3$ left as insoluble residue.

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# INTERACTION OF HYDROXYACETOPHENONES AND THEIR DERIVATIVES AND THIONYL CHLORIDE IN PRESENCE OF FINELY DIVIDED COPPER. PART VII. PREPARATION OF 3 : 3′-DIACETYL- AND 3 : 3′-DIPROPIONYL-4 : 4′-DIHYDROXY-5 : 5′-DIMETHYLDIPHENYL SULPHIDES AND THEIR DERIVATIVES

By V. G. Kulkarni and G. V. Jadhav

Preparation of 3 : 3′-diacetyl- and 3 : 3′-dipropionyl-4 : 4′-dihydroxy-5 : 5′-dimethyldiphenyl sulphides is described. Their diacetoxy derivatives and 2 : 4-dinitrophenylhydrazones as well as nitration products are also described.

Present work is the extension of the work of Jadhav and Merchant (*J. Univ. Bombay*, 1951, **19**, Part V, 41) and has been carried out with a view to studying the effect of the introduction of Me group in the nucleus of *o*-hydroxyacetophenone towards the reaction of thionyl chloride in presence of finely divided copper, as well as the effect of lengthening the chain attached to the ketonic group in that reaction. Introduction of Me group had practically no effect on the reactivity of the ketone, as the sulphide (I) was formed only when the reactants were allowed to react directly at a low temperature, similar to the case of simple *o*-hydroxyacetophenone.

Lengthening of the chain of the ketone deactivated the molecule further, as the reaction mixture required heating to complete the reaction.

Pure sulphides could be obtained with difficulty in both the cases, as described in the Experimental.

The same sulphide (I) was also obtained by the action of sulphur monochloride and sulphur dichloride on 2-hydroxy-3-methylacetophenone, and the sulphide (II) was prepared by the action of sulphur dichloride on 2-hydroxy-3-propiophenone in presence of copper.

The presence of -OH and -CO groups in the sulphides was proved by preparing their acetoxy and 2 : 4-dinitrophenylhydrazone derivatives.

The position of sulphur linking was located by nitrating the sulphides in presence of sulphuric acid, when both the sulphides furnished the same 2-methyl-4 : 6-dinitrophenol (3 : 5-dinitro-*o*-cresol, Me = 1). This showed that one nitro group came in place of the ketonic group and the other in place of the sulphur atom which had linked the nuclei. As the ketonic group is in the position-6 (-OH = 1), sulphur linking must be in the position-4, which is *para* to -OH group and *meta* to -CO group.

The replacement of the ketonic group by the nitro group must have taken place by its first being oxidised to -COOH and then this -COOH group was replaced by the nitro group, as such replacement of the COOH group by $NO_2$ group had already been reported by Hirve, Jadhav and Chakradeo (*ibid.*, 1934, **11**, 554), who obtained 2-methyl-4 : 5 : 6-trinitrophenol from 3 : 3′-dicarboxy-4 : 4′-dihydroxy-5 : 5′-dimethyldiphenyl sulphide.

The mechanism of the reaction may be explained in the same way as has been done by Hirve *et al.* (*J. Amer. Chem. Soc.*, 1935, **57**, 101).

## EXPERIMENTAL

*3 : 3′-Diacetyl-4 : 4′-dihydroxy-5 : 5′-dimethyldiphenyl Sulphide* (I).—Thionyl chloride (5 c.c.) was added to 2-hydroxy-3-methylacetophenone (3-acetyl-*o*-cresol, Me = 1) (3 c.c.) and copper powder (2 g.) was gradually added to it (over a period of 2 hours). The reaction was allowed to proceed overnight at room temperature, the mixture having been protected from moisture. It was then first washed with dry ether and then extracted with boiling benzene. On removal of the benzene a yellowish white paste remained, which solidified on treatment with acetone. It was finally crystallised from chloroform. It sintered at 155° and completely liquefied at 240-41°, yield 0.8 g. (Found : C, 65.5 ; H, 5.5 ; S, 9.5. $C_{18}H_{18}O_4S$ requires C, 65.5 ; H, 5.4 ; S, 9.7 per cent).

The same sulphide was obtained from the ketone (3 c.c.) and sulphur dichloride (3 c.c.) by interacting them on a boiling water-bath for 3 hours and then leaving the reaction mixture overnight at room temperature, and also by the interaction of the ketone (3 c.c.) and sulphur monochloride and leaving them overnight at room temperature.

*3 : 3′-Diacetyl-4 : 4′-diaceloxy-5 : 5′-dimethyldiphenyl sulphid.* was prepared by interacting the sulphide (I) and acetic anhydride in presence of pyridine. It crystallised from alcohol, m.p. 105-106°. (Found : S, 7.8. $C_{22}H_{22}O_6S$ requires S, 7.7 per cent).

The *2 : 4-dinitrophenylhydrazone* of the above diphenyl sulphide (I)was crystallised from chloroform, m.p. 187°. (Found : S, 4.8. $C_{30}H_{26}O_{10}N_8S$ requires S, 4.6 per cent).

*2-Methyl-4 : 6-dinitrophenol* (*3 : 5-Dinitro-o-cresol*).—The sulphide (I) (1 g.) was suspended in $H_2SO_4$ (20 c.c.) and $HNO_3$ (conc., 15 c.c.) was added to it when a clear yellowish solution was obtained. It was left overnight at room temperature and then diluted and extracted with ether. The solid obtained after removal of the ether was crystallised from alcohol, m.p. 86-87°. (Found : N, 13.9. Calc. for $C_7H_6O_5N_2$ : N, 14.1 per cent). Rapp (*Annalen*, 1884, **224**, 175) reports m.p. 85-88°. No lowering in melting point was observed when mixed with a genuine specimen.

The same dinitro-*o*-cresol was obtained when the sulphide (II) was treated with nitric acid in presence of sulphuric acid.

*3 : 3′- Diproprionyl-4 : 4′- dihydroxy-5 : 5′- dimethyldiphenyl Sulphide* (II).—Copper powder (2 g.) was gradually added to the mixture of 2-hydroxy-3-methylpropiophenone (3 c.c.) and thionyl chloride (5 c.c.). Half an hour after the addition of copper was over the mixture was refluxed on a boiling water-bath for 3 hours. When cold, the mixture was extracted with chloroform. The red paste obtained after removal of the solvent was repeatedly washed with petroleum ether and then subjected to the action of steam for ½ hour. When cooled, a red solid separated. It was finally crystallised from benzene, m.p. 98°. (Found : C, 67.1 ; H, 6.2 ; S, 9.1. $C_{20}H_{22}O_4S$ requires C, 67.0 ; H, 6.1 ; S, 8.9 per cent).

It is very soluble in acetone, chloroform, carbon disulphide, sparingly so in benzene, very sparingly in ether, acetic acid, and insoluble in alcohol and petroleum ether.

The same sulphide was obtained by allowing the ketone (3 c.c.), sulphur dichloride (2 c.c.) and copper powder (1 g.) to react overnight at room temperature.

3 : 3′-*Dipropionyl*-4 : 4′-*diacetoxy*-5 : 5′-*dimethyldiphenyl sulphide* was prepared by the action of acetic anhydride on the sulphide (II) in presence of pyridine. It crystallised from benzene, m.p. 75-76°. (Found : S, 7.0. $C_{24}H_{26}O_6S$ requires S, 7.2 per cent).

2 : 4-*Dinitrophenylhydrazone* of the above diphenyl sulphide (II) was crystallised from chloroform, m.p. 86-87°. (Found : S, 4.6. $C_{32}H_{30}O_{10}N_8S$ requires S, 4.5 per cent).

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# SALICYLAMIDOXIME AS AN ANALYTICAL REAGENT. PART III. SPECTROPHOTOMETRIC DETERMINATION OF URANIUM

By Debabrata Bandyopadhayay

The use of salicylamidoxime as a reagent for the spectrophotometric determination of uranium, by making use of the orange-yellow colour formed by uranium$^{VI}$ with the reagent in alkaline solution, is described. The optimum wave-length for measurement of the colour intensity is 400 mμ; the $p_H$ of the solution should be within the limit of 7.9-9.2, and an excess of the amidoxime, at least seven times the molar proportion of uranium, is essential. The colour is stable at room temperature and its intensity is independent of temperature within the range of 20°-25°, but measurements can safely be carried out at temperatures up to 30°. Beer's law holds good within the range of 7-145 p.p.m. of uranium, and sensitivity is 0.12 γ $cm^{-2}$. The composition (1 : 1) of the coloured species has been evaluated by Job's method and the probable constitution has been assigned Most cations and anions interfere with the determination of uranium. Hence, a preliminary separation of uranium from the interfering ions is essential as in most other cases

The colorimetric methods for uranium are not so sensitive as those of many other elements. Recently Yoe *et al.* (*Anal. Chem*, 1953, **25**, 1200) have proposed dibenzoylmethane, $C_6H_5.CO.CH_2.CO.C_6H_5$, as a colorimetric reagent for uranium, and it is the most sensitive reagent known so far. But the insolubility of the reagent in water is one great disadvantage. Of the various other substances that have been used for the colorimetric determination of uranium, mention may specially be made of salicylic acid (Müller, *Chem. Ztg.*, 1919, **43**, 739), sulphosalicylic acid (Rodden, "Analytical Chemistry of the Manhattan Project," 1st. Ed., p. 121, McGraw-Hill Book Co., Inc., 1950), salicylamide (Rây *et al.*, this *Journal*, 1953, **30**, 491), and salicylhydroxamic acid (Bhaduri, *Science & Culture*, 1952, **18**, 95). The relative merits of the common colorimetric procedures for uranium have also been investigated (Rây *et al*, *Proc. Indian Sci. Cong.*, 1954, Part III, p. 48).

It has already been stated in a previous communication (cf. Part I, Rây and Bandyopadhayay, this *Journal*, 1956, **33**, 21) that salicylamidoxime, $o$-$C_6H_4(OH).C(:NOH).NH_2$, developes an orange-yellow colour with uranium$^{VI}$ in alkaline solution, which is suitable for the detection of the metal. It has therefore been considered desirable to investigate the possibility of using this substance as a reagent for the spectrophotometric determination of uranium. The optimum conditions for such a procedure have all been determined and from the results the present method appears to be quite suitable. The optimum wave-length for measurement of the colour intensity appears to be 400 mμ, where the freshly prepared solution of the reagent has no absorption. The colour intensity in presence of an excess of the amidoxime remains constant at the maximum value in the range 7.9-9.2 $p_H$. Beer's law holds good over the range 7-145 p.p.m. of uranium and sensitivity (cf. Sandell, "Colorimetric Determination of Traces of Metals", 2nd Ed., p. 50, Interscience Publishers, Inc., 1950) is 0.12 γ $cm^{-2}$; while the experimental limit is 0.48 γ uranium per c.c. per cm.

The composition of the coloured product has been evaluated by Job's method (*Ann. chim.*, 1928, *x*, **9**, 113 ; 1936, *xi*, **6**, 97) of 'continuous variation' as modified by Vosburgh and Cooper (*J. Amer. Chem. Soc.*, 1941, **63**, 437 ; 1942, **64**, 1630) and the formation of an 1 : 1 complex between $UO_2^{2+}$ ion and the reagent has been indicated. Since the coloured species is insoluble in organic solvents like ether, chloroform, ethyl acetate, etc., but is highly soluble in water, it may reasonably be concluded (cf. Martell and Calvin, "Chemistry of the Metal Chelate Compounds", pp. 50-54, Prentice-Hall, Inc., 1952) that it is an ionic complex, possibly of the anionic type, as it is formed at a rather high $p_H$.

An attempt to determine the exact composition of the coloured species by $p_H$-titration method failed to furnish conclusive results, possibly owing to the fact that the complex was highly dissociated in solution, and did not provide a sharp break in the $p_H$-titration curve. The instability is apparent from the fact that a large excess of the reagent is necessary for the optical density of the solution to reach its maximum value, as has already been referred to.

Most common anions and cations interfere with the determination of uranium, although a few like $NO_3^-$, $Cl^-$, $SO_4^{2-}$ do not interfere at all, even when present in large amounts. The interference of cations is either due to colour-formation or precipitation of an insoluble complex or of the hydrated oxide. The approximate tolerance limits of most of the interfering ions have been determined and the results are recorded in Table III.

A previous separation of uranium from the interfering ions is necessary in this method of determination as in many other cases.

## Experimental

A Unicam SP 600 Spectrophotometer was used for the measurement of absorption of solutions using cells of suitable thickness (5 mm., 10 mm., or 20 mm., as required) in order to have the transmittancy values within the proper range (20-80%) for minimum error. Measurements of $p_H$ were carried out with the help of a Cambridge $p_H$-meter (Bench pattern) using glass electrode (for 0-9 $p_H$) or alkali-glass electrode (for $p_H$ 9 and over) and a saturated calomel reference electrode.

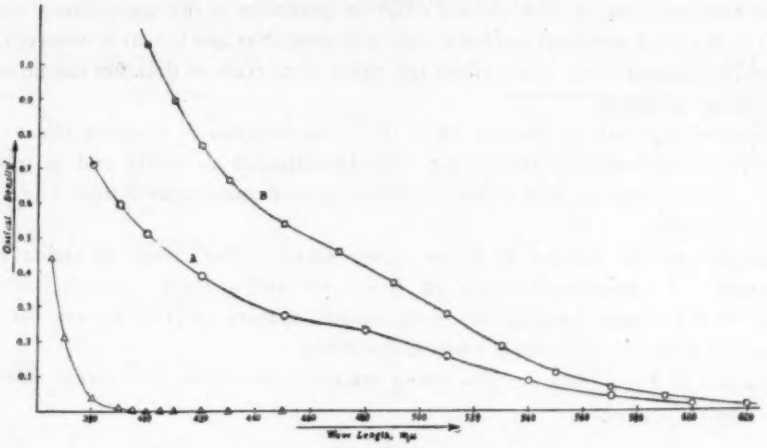
A freshly prepared solution of pure salicylamidoxime in water (vide Part I, *loc. cit.*) was always used for the measurements. A solution of uranyl nitrate was prepared by dissolving 5 g. of $UO_2(NO_3)_2$, $6H_2O$ (A.R., B.D.H.) in 500 c.c. of water containing some nitric acid, and standardised gravimetrically by precipitation with tannic acid and igniting the precipitate to $U_3O_8$. From this stock solution others of desired strengths were prepared by requisite dilution.

Stock solutions (0.1 *M* and 0.5 *M*) of caustic potash were prepared from G.R. quality KOH, and these were used for the adjustment of $p_H$ of the solutions. Solutions of diverse ions were prepared from suitable salts (reagent grade) and were standardised as usual. Phthalate and borate buffers (cf. Clark and Lubs, *J. Biol. Chem.*, 1916, **25**, 479) of $p_H$ 3.6 and 9.2 respectively, were used to set the $p_H$-meter during $p_H$ measurements.

*Determination of the Optimum Wave-length for Absorption Measurements.*—The absorption spectra of the coloured complex, in presence of excess of the amidoxime, at $p_H$ 4.97 and $p_H$ 8.72 are represented graphically in Fig. 1 along with

FIG. 1

*Absorption spectra* (25°).




A – $p_H$ 4.97 } $UO_2(NO_3)_2$ – 0.0005 *M* +
B—$p_H$ 8.72 } sal. amidoxime—0.005 *M*.
C—$p_H$ 8.22 ; sal. amidoxime—0.005 *M*.

that of the pure reagent of the same concentration at $p_H$ 8.22. From the figure it follows that the optimum wave-length for measurement is 400 mμ, where the pure reagent shows no absorption. This has therefore been used for subsequent measurements. That $p_H$ has no influence on the absorption of the reagent is indicated by the results recorded below.

TABLE I

Salicylamidoxime = 0.005 *M*. Temp. = 25°.

| $p_H$ | ... | 4.18 | 5.02 | 6.22 | 7.36 | 8.22 | 9.10 |
|---|---|---|---|---|---|---|---|
| Optical density per cm. (400 mμ) | ... | 0.002 | 0.003 | 0.002 | 0.002 | 0.002 | 0.002 |

*Stability of the Reagent Solution.*—Stock solutions (0.01*M*) of salicylamidoxime were prepared at different $p_H$ values by adding acid ($H_2SO_4$) or alkali (KOH) as necessary and stored for several days. The optical density of each solution was measured at intervals of a day or two after adjusting the $p_H$ of the requisite aliquot to about 8.5 and then diluting to the required volume to give a 0.005 *M* solution. The aqueous and alkaline solutions were found to be unstable, deteriorating readily on storing. But the acid solutions were much more stable. Thus, a 0.01*M* solution of the amidoxime at a $p_H$ of 2.52 after being stored for a month and then examined as before showed an optical density of 0.022 per 2 cm.

*Effect of $p_H$ on the Colour Intensity.*—Solutions containing the same concentration of uranium and the same excess of the amidoxime were prepared at different $p_H$ values and their absorption measured at 400 mμ. The results show that the colour intensity reaches its maximum and is independent of the $H^+$-ion concentration within the range 7.9 to 9.2 $p_H$.

*Influence of Reagent Concentration on the Colour Intensity.*—Solutions containing the same concentration of uranium and different quantities of the amidoxime were prepared at $p_H$ 8.5±0.1 and their optical densities measured at 400 mμ. It is observed that an excess of the reagent over seven times the molar proportion of uranium has no influence on the colour intensity.

*Variation of Optical Density with the Concentration of Uranium (Beer's law).*—Conformity of the system to Beer's law was investigated as usual and it was found that the system obeyed Beer's law in the range of concentration from 7.14 to 145.18 p.p.m. of uranium.

*Stability of the Colour at Room Temperature.*—The colour is stable at room temperature. A solution 0.0005*M* in $UO_2^+$ ion and 0.005*M* in salicylamidoxime at a $p_H$ of 8.52 gave exactly the same optical density of 1.06 per cm., at 400 mμ, even after 72 hours of standing at room temperature.

*Influence of Temperature.*—The colour intensity was found to decrease appreciably over 30°, as shown below.

TABLE II

$UO_2^{2+}$ = 0.0005*M*. Salicylamidoxime = 0.0025*M*. $p_H$ = 8.22.

| Temp. ... | 20° | 25° | 30° | 35° | 40° | 45° |
|---|---|---|---|---|---|---|
| Optical density per cm. (400 mμ) .... | 0 530 | 0.528 | 0.520 | 0.510 | 0.500 | 0 490 |

*Sensitivity.*—Solutions containing 0.238, 0.476 and 0.714 p.p.m. of uranium showed optical densities (at 400 mμ) of 0.000, 0.008 and 0.013 per 2 cm. Hence, the corresponding values per cm. are 0.000, 0.004, and 0.007 respectively. Therefore, the smallest amount of uranium that can still be detected with the instrument is 0.48 γ per c.c. per cm. while, the spectrophotometric sensitivity (cf. Sandell, *loc. cit.*) is 0.12 γ $cm.^{-2}$ of uranium.

*Composition of the Coloured Product.*—This was determined by following the method of continuous variation, first employed by Job (*loc. cit.*, cf. Vosburgh and Cooper, *loc. cit.*). In order to prevent the precipitation of uranium in solutions containing lesser amounts of salicylamidoxime (R), it was found necessary to work with very dilute solutions ($[UO_2^{2+}]+[R]=4\times10^{-4}M$ in actual experiments). At this extremely low concentrations the absorption of the free $UO_2^{2+}$ ion was found to be negligible at the wave-lengths (400 mμ & 420 mμ; used in the investigations, while the reagent did not show any absorption at these wave-lengths (cf. Fig. 1). Hence, a plot of the observed optical density against the composition of the solution, *i.e.* $[R]/([R]+[UO_2^{2+}])$, furnished the composition curves represented in Fig. 2. These pass through a maximum at a composition corresponding to 0.5, indicating the formation of an 1 : 1 complex between $UO_2^{2+}$ ion and salicylamidoxime.

*Influence of Foreign Ions on the Uranyl-salicylamidoxime Colour.*—The effect of diverse ions was studied as usual and the approximate tolerance limits were determined. The latter denotes the maximum permissible concentration of the foreign ion in the solution under investigation which will affect the optical density by less than ±2%. The results are shown in Table III.

TABLE III

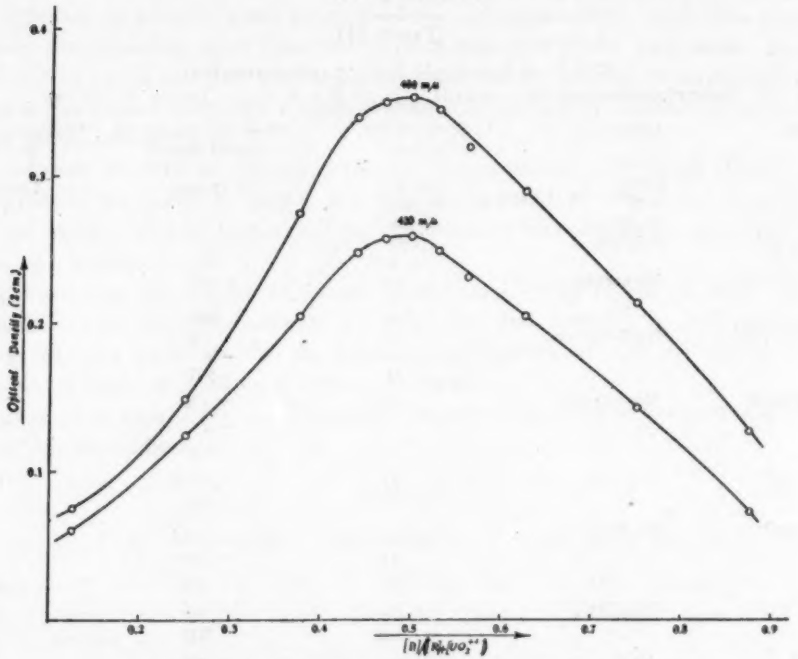
$UO_2^{2+}$ = 0.00025*M* (59.5 p.p.m. uranium).
Salicylamidoxime = 0.0025*M*. $p_H$ = 8.5 ± 0.3. Temp. = 20°-25°.

| Ion. | Added as. | Conc. of ion (p.p.m.) | Observed change in optical density. | Tolerance limit (p.p m.). |
|---|---|---|---|---|
| $NO_3^-$ | $KNO_3$ | 16 000 | No change | Large excess |
| $Cl^-$ | KCl | 7,000 | ,, | ,, |
| $SO_4^{2-}$ | $Na_2SO_4$ | 24,000 | ,, | ,, |
| $C_2O_4^{2-}$ | $Na_2C_2O_4$ | 20 | − 2% | 10 |
| | | 4 | Nil | |
| $CH_3COO^-$ | Na-acetate | 30 | − 2% | |
| | | 15 | − 1 | 20 |
| | | 6 | Nil | |
| Tartrate | Na-K-tartrate | 60 | − 5% | |
| | | 30 | − 2 | 20 |
| | | 15 | Nil | |
| Citrate | $Na_3$-citrate | 16 | − 5% | |
| | | 8 | − 2 | 6 |
| | | 4 | Nil | |
| $CO_3^{2-}$ | $Na_2CO_3$ | 24 | − 2.6% | 15 |
| | | 6 | Nil | |
| $BO_3^{3-}$ | $Na_2B_4O_7$ | 24 | − 7.5% | |
| | | 12 | − 3.6 | |
| | | 6 | − 1.6 | 6 |
| $PO_4^{3-}$ | $Na_2HPO_4$ | 12 | − 1.6 | 12 |
| | | 6 | Nil | |
| $F^-$ | NaF | 135 | − 2% | |
| | | 90 | − 1 | 100 |
| | | 45 | Nil | |
| $MoO_4^{2-}$ | $Na_2MoO_4$ | 200 | + 1.6% | 200 |
| $WO_4^{2-}$ | $Na_2WO_4$ | 170 | + 4 | |
| | | 110 | + 2 | 80 |
| | | 11 | Nil | |
| $VO_3^-$ | $NH_4VO_3$ | 10 | + 3% | |
| | | 5 | + 2 | 3 |
| $Cr_2O_7^{2-}$ | $K_2Cr_2O_7$ | 2 | + 16 | 0 |
| $Bi^{3+}$ | $Bi(NO_3)_3$ | 2 | Turbid | 0 |
| $Cu^{2+}$ | $CuCl_2$ | 1 | ,, | 0 |
| $Ni^{2+}$ | $NiCl_2$ | 1 | ,, | 0 |
| $Co^{2+}$ | $CoCl_2$ | 3 | + 8% | |
| | | 1 | + 3 | 0 |
| $Mn^{2+}$ | $MnCl_2$ | 2 | + 10 | 0 |
| $Cr^{3+}$ | $CrCl_3$ | 2 | + 8 | 0 |
| $Fe^{3+}$ | $FeCl_3$ | ... | Interferes at all concentrations | 0 |
| $Ce^{4+}$ | $Ce(NO_3)_4$ | 1 | Turbid | 0 |
| $Th^{4+}$ | $Th(NO_3)_4$ | 1 | ,, | 0 |
| $Ti^{4+}$ | $TiO(SO_4)$ | 1 | ,, | 0 |

*Procedure.*—From a consideration of the experimental results recorded above, the following procedure for the estimation of uranium by salicylamidoxime may be recommended.

FIG. 2

*Job's method.*




$UO_2^{2+}$—salicylamidoxime ($p_H$ = 8.6 ± 0.1; temp. = 24°).

For the determination of uranium in commercial materials, ores, minerals, etc., a solution of the sample should be prepared and treated to separate the other elements present as usual (cf. Snell and Snell, "Colorimetric Methods of Analysis", Vol. II, pp. 490-91, D. Van Nostrand Co., Inc., 1949; Sandell, "Colorimetric Determination of Traces of Metals", 2nd Ed., pp. 595-96, Interscience Publishers, Inc., 1950). The pure uranyl salt solution, as chloride, nitrate or sulphate, thus obtained, should be treated directly or in an aliquot portion with an excess (over seven times the molar proportion of uranium) of a freshly prepared solution of pure salicylamidoxime in water or dilute acid (HCl). The $p_H$ of the solution is to be adjusted to about 8.5-9.0 by adding alkali (KOH) and then the solution diluted to a requisite volume such that the uranium content lies between 12 and 145 p.p.m.; the $p_H$ of the final solution should be within the limit of 8.0-9.0. The optical density of the solution can then be read at 400 mμ in a cell of suitable thickness (2cm., 1cm., or 0.5 cm.) to have the optimum transmission, and the temperature should preferably be within the range of 20° to 25°. From the optical density value the concentration of uranium can be calculated from the known value of the extinction coefficient, since Beer's law is obeyed in the prescribed range of uranium concentration.

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# SALICYLAMIDOXIME AS AN ANALYTICAL REAGENT. PART IV. SPECTROPHOTOMETRIC DETERMINATION OF IRON

By Debabrata Bandyopadhayay

The use of salicylamidoxime as a reagent for the spectrophotometric determination of iron, by making use of the orange-red colour formed by $Fe^{III}$ with the reagent in alkaline solution, is described. The optimum wave-length for measurement of the colour intensity appears to be 400 mμ, since the absorption of the coloured complex shows very little change within the range 390-410 mμ and the freshly prepared reagent shows no absorption in this region. The $p_H$ of the solution should be within the limit of 8.3 to 10.0 and an excess of the reagent, at least twenty times the molar proportion of iron, is essential. The colour is stable at room temperature in the $p_H$ range mentioned and measurements can be carried out within the range of 20° to 30°. Beer's law holds good within the range 0.28 to 14 p.p.m. of iron, and sensitivity is 0.01 $\gamma$ $cm^{-2}$. The composition (1 : 3) of the coloured species has been evaluated by Job's method and a probable constitution has been assigned. The effect of diverse ions on the colour intensity has been investigated and the tolerance limits have been determined. This method therefore requires a previous separation of iron from the interfering radicals as in most other cases.

The colorimetric reagents for iron are fairly numerous, but comparatively few are well suited for the determination of traces of this metal. Of the various reagents employed some react with ferrous ions and some with ferric ions. The latter generally have the disadvantage that they cannot be used in the presence of ions like fluoride, phosphate, pyrophosphate, etc., which strongly mask $Fe^{III}$ in acid solution in which the colour reactions have frequently to be carried out. Determinations of $Fe^{III}$ in alkaline solutions, however, can often be carried out even in presence of appreciable quantities of these otherwise interfering ions.

It has already been pointed out in Part I (Rây and Bandyopadhayay, this *Journal*, 1956, **33**, 21) that ferric ion gives a strongly coloured soluble complex with salicylamidoxime in alkaline solution which is suitable for the detection of traces of iron. An attempt to use the amidoxime as a reagent for the determination of traces of iron seems desirable since reagents which react in alkaline solution have usually some advantage, as already mentioned. As a result of the present investigation it has been found that the amidoxime is quite useful for the colorimetric determination of iron. The optimum wave-length for measurement of the colour intensity appears to be 400 mμ, since the absorption of the coloured complex shows very little change in the region 390-410 mμ, and the freshly prepared solution of the reagent shows no absorption at this wave-length. The colour intensity of a solution containing $Fe^{III}$ and excess of the amidoxime reaches its maximum at a $p_H$ 8.3 and remains constant up to $p_H$ 10. An excess of the reagent over twenty times the molar proportion of iron is without effect on the colour intensity, and Beer's law has been found to hold good within the entire range investigated, *i.e.*, 0.28 to 14 p.p.m. of iron. Sensitivity of the present method is 0.01 $\gamma$ $cm^{-2}$, and as little as 0.05$\gamma$ Fe per c.c. per cm. can be detected with the instrument. The colour is stable at room temperature and the optical density remains unaltered in the range of 20°-30°, and even at 40° the decrease is less than 2%.

The composition of the coloured complex has been evaluated by Job's method as in the case of the $U^{VI}$ complex, described in Part III (this *issue*, p. 269). The results indicate the formation of an 1 : 3 complex between the ferric ion and salicylamidoxime. The fact that the colour is highly soluble in water and cannot be extracted by organic solvents indicates its ionic nature (cf. Part III, *loc. cit.*).

The effect of diverse ions on the colour intensity has been fully investigated. Ions like $NO_3^-$, $Cl^-$, $SO_4^{2-}$, $BO_3^{3-}$ do not interfere even when present in large excess; oxalate, tartrate, $SCN^-$ and $F^-$ interfere when present in sufficient amounts, while most other ions interfere even in low concentrations.

The present method is highly sensitive, but a previous separation of iron from most other cations and anions is necessary before it can be applied to ores, alloys, etc., as in most other cases.

## Experimental

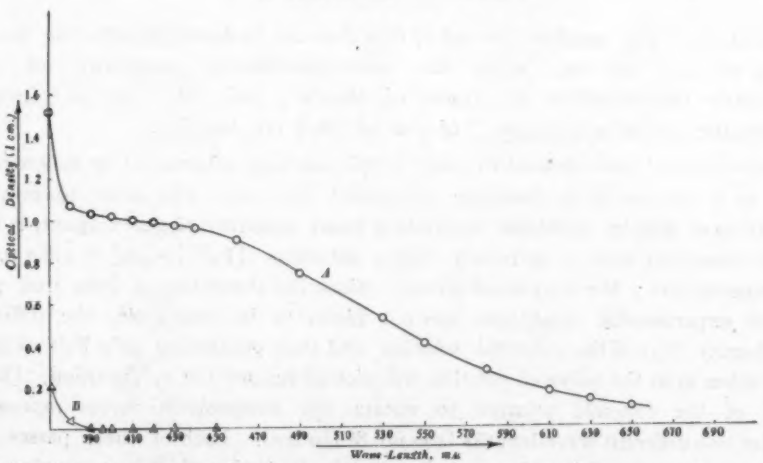
The apparatus and solutions of reagents, alkali, diverse ions, buffers, etc., actually used, are the same as those described in Part III (*loc. cit.*).

*Ferric Nitrate Solution.*—A stock solution of ferric nitrate was prepared from $Fe(NO_3)_3$, $9H_2O$ (G.R.- E. Merck) and was standardised gravimetrically as the oxide and volumetrically against potassium dichromate as usual. From this stock solution others of required strengths were prepared by requisite dilution with double-distilled water, containing some nitric acid to prevent the hydrolysis of the salt.

*Determination of the Optimum Wave-length for Absorption Measurements.*—The absorption spectrum of the coloured complex in presence of an excess of salicyl-

Fig. 1

*Absorption spectra at 23°.*




Curves A and B refer respectively to 0.00025-*M*-$Fe(NO_3)_3$ +0.005 *M*-sal.-amidoxime ($p_H$ 9.14) and 0.005 *M*-salicylamidoxime,

amidoxime at a $p_H$ of 9.14 and of the reagent of the same concentration and nearly the same $p_H$ as that of the coloured solution are represented in Fig. 1, from which it follows that the optimum region for measurement of the colour intensity is 400 mμ.

*Effect of $p_H$ on the Colour Intensity.*—The results show that the colour intensity at 400 mμ reaches its maximum and is independent of $H^+$-ion concentration within the range 8.3-10.0 $p_H$.

*Influence of Reagent Concentration on the Colour Intensity.*—It is observed that an excess of reagent over twenty times the molar proportion of iron is without effect on the colour intensity. Measurements have been made at $p_H$ 8.8 ± 0.1 and 400 mμ.

*Variation of Optical Density with the Concentration of Iron (Beer's law).*—Results of experiments show that Beer's law is obeyed in the entire range of concentrations investigated, namely 0.28 to 14 p.p.m. of iron. The practical range for measurements in 1 cm. cell is, however, 1.4 to 9.5 p.p.m. of iron so that the transmission lies within the optimum range of 80-20% (optical density 0.1-0.7).

*Stability of the Colour at Room Temperature.*—The colour is stable at room temperature. Thus, a solution 0.002*M* in $Fe^{3+}$ and 0.002*M* in salicylamidoxime at a $p_H$ of 8.94, gave exactly the same optical density of 0.409 per cm. at 400 mμ, even after 24 hours of standing at room temperature (25°).

*Influence of Temperature.*—The colour intensity remains unaltered within the range of 20°-30°; further increase of temperature leads to a slight and gradual decrease in the optical density of the solution as shown below.

TABLE I

$Fe^{3+}$ = 0.0001 *M*. Salicylamidoxime = 0.004 *M*. $p_H$ = 8.87.

| Temp. | 20° | 25° | 30° | 35° | 40° | 45° |
|---|---|---|---|---|---|---|
| Optical density per cm., at 400 mμ | 0.410 | 0.410 | 0.408 | 0.406 | 0.403 | 0.401 |

*Sensitivity.*—The smallest amount of iron that can be detected with the instrument is 0.05 γ per c.c. per cm., while the spectrophotometric sensitivity (cf. Sandell, "Colorimetric Determination of Traces of Metals", 2nd Ed., p. 50, Interscience Publishers Inc., 1950) is 0.01γ cm.$^{-2}$ of iron (cf. Part III, *loc.cit.*).

*Composition of the Coloured Product.*—This has been determined by following Job's method as in the case of the uranium compound (*loc. cit.*). In order to prevent the precipitation of iron in solutions containing lesser amounts of the reagent, it has been found necessary to work in extremely dilute solutions ($[Fe^{3+}] + [R] = 1.6 \times 10^{-6}$ *M* in actual experiments; R = salcylamidoxime). Since the absorption of pure iron solutions under the experimental conditions was not found to be negligible, the difference in optical density ($D_\Delta$) of the coloured solution and that containing only $Fe^{III}$ of the same concentration as in the coloured solution was plotted against the composition, $[R]/([R] + [Fe^{3+}])$, of the coloured solution to obtain the composition curves represented in Fig. 2 for two different wave-lengths (400 mμ & 450 mμ). Each of these passes through a maximum at a composition of 0.75, indicating the formation of an 1:3 complex between $Fe^{III}$ and salicylamidoxime.

*Influence of Foreign Ions on the* $Fe^{III}$*-salicylamidoxime Colour.*—The effect of diverse ions has been studied and the tolerance limits have been determined as in case of the colour system produced by $U^{VI}$ (*loc. cit.*). The results are presented in Table II.

TABLE II

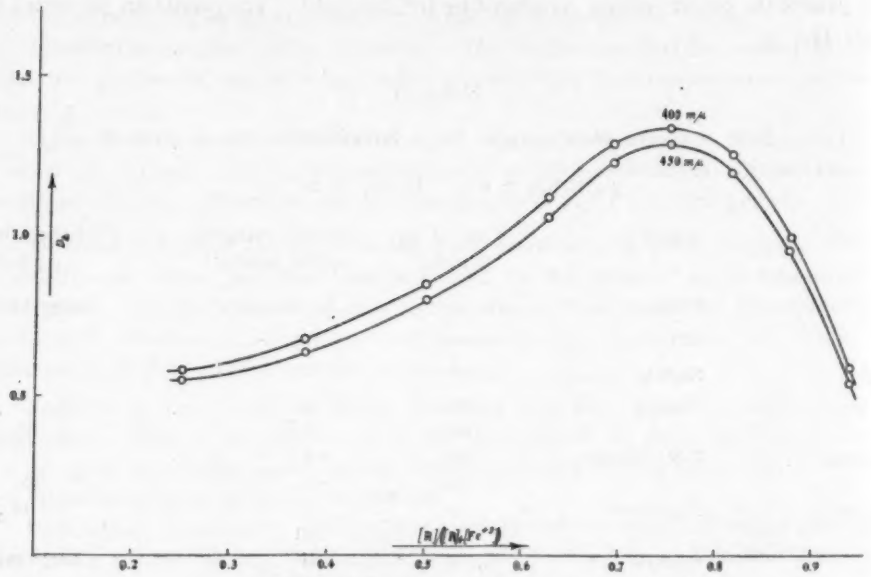
$Fe^{3+}$ = 0.0001 *M* (5.6 p.p.m. Fe). Salicylamidoxime = 0.002 *M*.

$p_H$ = 8.9 ± 0.2. Temp. = 20°-25°.

| Ion. | Added as. | Conc. of ion (p.p.m.) | Observed change in optical density. | Tolerance limit (p.p.m.). |
|---|---|---|---|---|
| $NO_3^-$ | $KNO_3$ | 12, 000 | No change | Large excess |
| $Cl^-$ | KCl | 10, 000 | ,, | ,, |
| $SO_4^{2-}$ | $Na_2SO_4$ | 14, 000 | ,, | ,, |
| $C_2O_4^{2-}$ | $Na_2C_2O_4$ | 600 | −4% | |
| | | 200 | −1.2 | 250 |
| Tartrate | K-Na-tartrate | 1, 400 | −4.5 | |
| | | 280 | −1.3 | 300 |
| Citrate | $Na_3$-citrate | 30 | −4 | 10 |
| | | 3 | Nil | |
| $BO_3^{3-}$ | $Na_2B_4O_7$ | 5, 000 | Nil | Large excess |
| $PO_4^{3-}$ | $Na_2HPO_4$ | 20 | −5% | |
| | | 10 | −2 | 6 |
| $F^-$ | NaF | 200 | −1.5 | 200 |
| $SCN^-$ | KSCN | 2, 400 | +4 | |
| | | 1, 200 | +2 | 1, 000 |
| $CO_3^{2-}$ | $Na_2CO_3$ | 6, 000 | −2.5 | |
| | | 3, 000 | −1.2 | 4, 000 |
| $MoO_4^{2-}$ | $Na_2MO_4$ | 400 | +3 | |
| | | 200 | +1.4 | 200 |
| $WO_4^{2-}$ | $Na_2WO_4$ | 500 | +3 | |
| | | 200 | +1.3 | 200 |
| $VO_3^-$ | $NH_4VO_3$ | 4 | +2.2 | |
| | | 2 | +1.2 | 2 |
| $UO_2^{2+}$ | $UO_2(NO_3)_2$ | ... | Interferes at all concentrations | 0 |
| $Cr_2O_7^{2-}$ | $K_2Cr_2O_7$ | 2 | +20% | 0 |
| $Bi^{3+}$ | $Bi(NO_3)_3$ | 2 | Turbid | 0 |
| $Cu^{2+}$ | $CuCl_2$ | 1 | ,, | 0 |
| $Ni^{2+}$ | $NiCl_2$ | 1 | ,, | 0 |
| $Co^{2+}$ | $CoCl_2$ | 3 | +2.4% | 0 |
| $Mn^{2+}$ | $MnCl_2$ | 1.2 | +3.8 | 0 |
| $Cr^{3+}$ | $CrCl_3$ | 2 | +10 | 0 |
| $Ce^{4+}$ | $Ce(NO_3)_4$ | 1 | Turbid | 0 |
| $Th^{+}$ | $Th(NO_3)_4$ | 1 | ,, | 0 |
| $Ti^{4+}$ | $TiO(SO_4)$ | 1 | ,, | 0 |

FIG. 2

*Job's method* (25°).




$Fe^{3+}$—salicylamidoxime ($p_H$ 9.0 ± 0.1).

*Procedure.*—The iron solution should be in the form of nitrate, chloride or sulphate, and all the iron must be in the ferric state. Interfering ions should be removed by suitable methods as commonly used. An aliquot of the solution should be mixed with at least over twenty times the molar proportion of the pure reagent. It is necessary to add the iron solution to a freshly prepared alkaline solution of the reagent and then adjust the $p_H$ to about 9 by adding acid or alkali as required ; the solution is now diluted to a definite volume, and its optical density determined at 400 mμ in a cell of suitable thickness to get the transmission within the optimum range for minimum error. The temperature of the solution should be between 20° and 30° and the concentration of iron should lie preferably between 0.7 and 14 p.p.m. From the observed optical density, the amount of iron present can be calculated easily, since the system obeys Beer's law.

The author wishes to acknowledge gratefully his indebtedness to Professor Priyadaranjan Rây, Professor of Inorganic Chemistry, Indian Association for the Cultivation of Science, for his kind help and keen interest during the course of this work.

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# REACTION OF GLYCEROPHOSPHORIC ACID AND ORTHOPHOSPHORIC ACID WITH ISOELECTRIC GELATIN STUDIED BY PHYSICO-CHEMICAL METHODS

By (Miss) Mira P. Gidwani and C. S. Narwani

The amount of either glycerophosphoric acid or orthophosphoric acid, bound with 1 g. of isoelectric gelatin at 37°, is found to be $80.40 \times 10^{-5}$ g. mol., as determined from the minima of the plateau of the graphs obtained by plotting bound $H^+$ ions against the equilibrium $p_H$ value of the mixture of gelatin sol and the solutions of the respective acids of concentrations, 0.002-0.016 *M*; this combination corresponds to 0.01*M* initial concentration of either acid, at which both are found to be ionised as monobasic acids. The conductometric titration and viscosity curves also show inflections at points corresponding to 0.01*M* initial concentration. In addition to chemical combination of both the acids with gelatin, there is also adsorption of the acid molecules, side by side, at $p_H$ value below 2.73 in the case of glycerophosphoric acid and below 2.8 in the case of orthophosphoric acid

In the formation of protein salts with strong acids, the anion of the acid may be co-ordinately bound at the $NH_3$ groups, forming $NH_3X$, in which one H has a four-electron shell as in $HF_2$, according to Phillips (*J. Int. Soc. Leather Trades Chem.*, 1933, **17**, 15, 69). Schellaman (*J. Phys. Chem.*, 1953, **57**, 472) attributes the binding of anions to proteins to the low dielectric constant of protein film sheathing by Coulomb's force. Pauli and Modern (*Biochem. Z.*, 1925, **156**, 482) observed that only acids with ionisation constants greater than that of the carboxyl groups of the proteins (*i.e.* with a value for $pk=4$) would be able to form salts with the proteins. In the case of orthophosphoric acid, which ionised in three stages [(1) $K' = 0.8 \times 10^{-2}$; (2) $K'' = 2.0 \times 10^{-7}$; (3) $K''' = 3.6\ 10^{-13}$] it was anticipated by Loeb (*J. Gen. Physiol.*, 1918-19, **1**, 39, 327, 559) and Hitchcock (*ibid.*, 1922, **4**, 733), but not verified, that like HCl and $H_2C_2O_4$, it would react with proteins as a monobasic acid. The object of the present work is to verify experimentally the above mentioned statement by studying potentiometric and conductometric titrations and viscosity changes of 1% aqueous solutions of isoelectric gelatin in presence of 0.2-1.6 m. mol./100 c.c. (corresponding to 0.002-0.016 *M* solutions) of orthophosphoric acid and glycerophosphoric acid.

## Experimental

All the chemicals used were of Merck's chemically pure quality. Isoelectric gelatin supplied by M/s. Kodak Ltd., London, having an ash value of 0.025% and giving an aqueous solution of $p_H$ 4.66 at 37°, was used; $CO_2$-free distilled water ($p_H$ 6.8) was used for preparing the solutions of the gelatin and the acids.

All measurements of physical properties were carried out in a thermostat with a toluene-mercury thermoregulator at 37° ±0.1°.

Ostwald's viscometer was used for viscosity measurements; Kohlrausch's bridge, for measuring electrical conductivity; Hydrogen electrode (Bunker's type), to measure the $p_H$ value of the solutions; and a potentiometer, reading to 0.2 millivolts, was used for measuring the E.M.F.

By studying the influence of dilution on the $p_H$ value of the aqueous solutions of glycerophosphoric and orthophosphoric acids, it was found that the former ionised as monobasic up to 0.01 *M* and dibasic up to 0.002 *M*, while the latter as monobasic up to 0.008 *M* and dibasic up to 0.002 *M*.

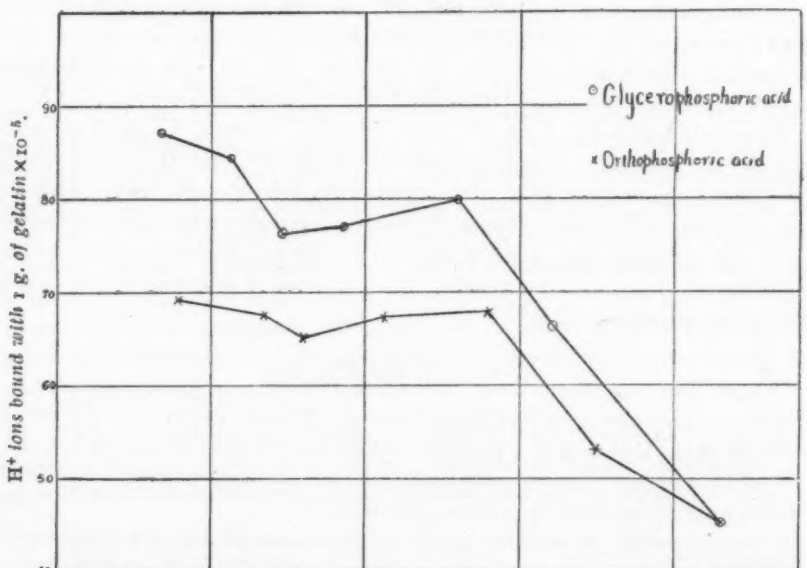
The results of potentiometric titrations of 1% isoelectric gelatin solution against glycerophosphoric and orthophosphoric acids respectively are shown in Table I ; 50 c.c. of glycerophosphoric acid or orthophosphoric acid of various molarities (0.004 to 0.032) were diluted to 100 c.c. with (1) $CO_2$-free distilled water and (2) 2% isoelectric gelatin solution; the $p_H$ values in the two cases were determined and the difference in $H^+$ ion concentration in 100 c.c. represented the amount of $H^+$ ions bound to 1 g. of gelatin.

$$\text{No. of g. equiv. of the acid bound} = \frac{\text{amt. of } H^+ \text{ ions bound}}{\text{degree of ionisation}};$$

$$\text{No. of g. mol. of the bound acid} = \frac{\text{No. of g. equiv. bound}}{\text{basicity of the acid}}.$$

The plots of $H^+$ ions bound with 1 g. of gelatin against the $p_H$ values of the mixture of 1% gelatin solution and various amounts of the acids are shown in Fig. 1.

FIG. 1




The conductometric titration curves for the two acids and the viscosity changes, represented by $\rho \times t$, where $\rho$=sp. gr. and $t$= time of flow, on addition of various amounts of the acid to 1 g. of gelatin are shown in Fig. 2.

TABLE I

*Potentiometric titration of* 100 c.c. *of* 1% *gelatin soln. against glycerophosphoric acid and orthophosphoric acid at* 37°.

| Acid (m mol./100 c.c. aq. soln). | Degree of ionisation of the acid. | $p_H$ of the aq. soln. of the acid. | $[H]\times10^5$ of the aq. soln. | $p_H$ of the mixture of the acid & gelatin soln. | $[H]\times10^5$ of the mixture. | Amt. of H ions bound $\times 10^5$. | G. mol. of the bound acid $\times10^5$. |
|---|---|---|---|---|---|---|---|
| | | | (1) Glycerophosphoric acid. | | | | |
| | Dibasic. | | | | | | |
| 0.2 | 1.00 | 2 33 | 460 | 4.15 | 7.08 | 45.29 | 22.64 |
| 0 4 | 0 86 | 2.16 | 690 | 3.61 | 24 55 | 66.54 | 38 68 |
| 0.6 | 0.71 | 2.07 | 850 | 3.30 | 50.12 | 79 98 | 56.32 |
| 0.8 | 0.56 | 2.05 | 890 | 2.93 | 117.50 | 77.20 | 68.92 |
| | Monobasic. | | | | | | |
| 1.0 | 0.95 | 2.02 | 950 | 2.73 | 186.20 | 76.38 | 80.40 |
| 1.2 | 0.92 | 1 95 | 1120 | 2.56 | 275.48 | 84.45 | 88.53 |
| 1.6 | 0 84 | 1 87 | 1350 | 2 33 | 467.70 | 87.23 | 103.84 |
| | Dibasic. | | (2) Orthophosphoric acid. | | | | |
| 0.2 | 1.00 | 2.33 | 460 | 4.15 | 7.08 | 45.29 | 22.64 |
| 0.4 | 0.83 | 2.19 | 650 | 3 75 | 17.78 | 53.22 | 30.85 |
| 0.6 | 0.62 | 2.14 | 720 | 3 40 | 39.81 | 68 02 | 58.85 |
| | Monobasic. | | | | | | |
| 0.8 | 0.95 | 2.12 | 760 | 3.06 | 87 10 | 67.29 | 70.83 |
| 1.0 | 0.81 | 2.09 | 810 | 2 80 | 158.50 | 65.15 | 80 43 |
| 1.2 | 0.76 | 2 05 | 890 | 2.67 | 213.81 | 67.62 | 88 97 |
| 1.6 | 0.69 | 1.96 | 1100 | 2.39 | 407.40 | 69 26 | 101.82 |

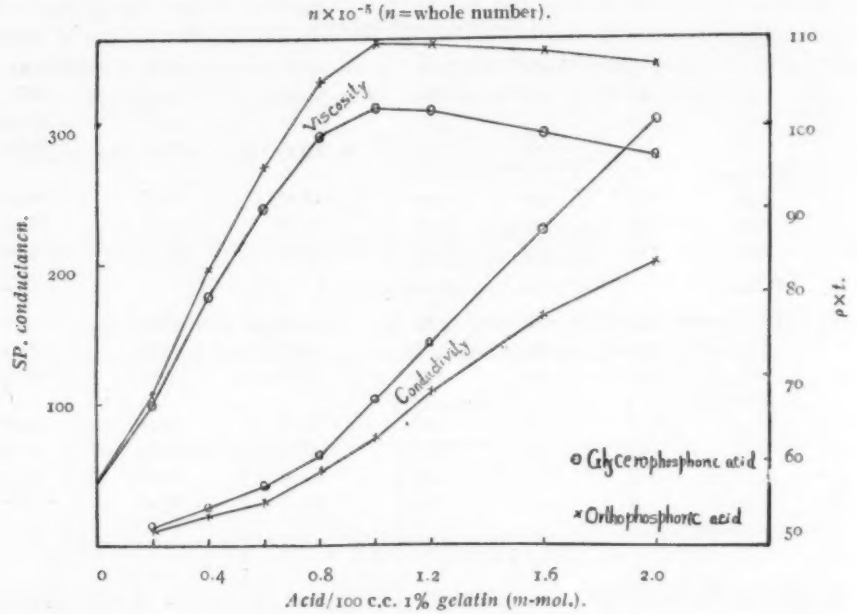
## DISCUSSION

On plotting combined $H^+$ ions against the equilibrium $p_H$ values of the mixtures of glycerophosphoric acid and gelatin, the curve shown in Fig. 1 has a minima in the plateau at $p_H$ 2.73, corresponding to $76.38\times10^{-5}$ g. equiv. of $H^+$ ions bound to 1 g. of gelatin. In case of orthophosphoric acid, the curve obtained is similar and has a minima in the plateau at $p_H$ 2.8, corresponding to $65.15\times10^{-5}$ g. equiv. of $H^+$ ions bound to 1 g. of gelatin. Both these $p_H$ values correspond to the same initial concentration of the two acids, 0.01 *M*. Again, the amount of the g. mols. of the acid bound to 1 g. of gelatin in the case of either acid is exactly the same, $80.4\times10^{-5}$. The g. eq. weight of gelatin, calculated from this value on the basis of the combination of the acids as monobasic, is 1243.6. The amount of $H^+$ ions bound being different while that of the acid bound being the same, it may be concluded that there is molecular rather than ionic combination between gelatin and the acids. In case of gelatin and hydrochloric acid a similar combination curve was obtained by Atkin and Douglas (*J. Int. Soc. Leather Trades Chem.*, 1924, **8**, 359). This shows that the phosphoric acids behave like strong acids at high dilution.

After the complete combination, the continuous increase in the amount of combined acid with increase in initial concentration of the acid may be attributed to adsorption of molecules of the acid to form adsorption complexes as concluded by Sinclair and Gortner (*Cereal Chem.*, 1933, **10**, 171) in case of gliadin and acetic acid.

Fig. 2 shows that in presence of 1% gelatin solution, the specific conductivity increases gradually with *increase* in concentration of glycerophosphoric acid up to 0.008 *M* and of orthophosphoric acid up to 0.01 *M*, after which there is a sudden increase and inflection in the curve; this means that the combination is complete at these concentrations which are the same as in the case of the potentiometric titration.

FIG. 2




The plots of $\rho \times t$ against concentration of the acids added to 1% gelatin solution show that viscosity goes on increasing rapidly with concentration of the acid added up to 0.01*M* and then decreases gradually. The increase in viscosity can be attributed to the increase in the production of positively charged gelatin ions on combination with the acid. In addition to electro-viscous effect, according to Gortner (*J. Phys. Chem.*, 1939, **43**, 721) the ions are hydrated and consequent increase in diameters retards their flow. On comparing Figs. 1 and 2 and referring to Table I, it is observed that the $p_H$ corresponding to maxima of viscosity curve coincides nearly with that of the minima of plateau of the combination curve.

On comparing the plots of viscosity and electrical conductivity (Fig. 2) it is observed that there is a reversal behaviour of specific conductance with respect to viscosity in case of the two acids; this is in accordance with the equation derived by Krasny-Ergen (*Kolloid Z.*, 1936, **74**, 172) in which the specific viscosity of the solution varies *inversely* as its specific conductance. It has been verified experimentally that the ionic conductivity of glycerophosphoric anion is greater than that of the orthophosphoric anion.

The authors take this opportunity of thanking M/s. Kodak Ltd., London for supplying free of charge, the isoelectric gelatin, used in the work.

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# THE REACTIVITY OF SULFHYDRYL GROUPS IN PROTEINS. PART I. AMANDIN

By R. Lontie and G. Beckers

Silver, *p*-chloromercuribenzoate and ferricyanide were allowed to react on amandin, both native and denatured. Native amandin possesses one "sluggish SH group" per molecule. No "masked SH group" becomes available after denaturation in 7.5 *M*-urea. Ferricyanide is not specific for the SH groups in all proteins.

Native amandin is reported to possess no SH group. Yet the only reagents used to detect them have been oxidising agents: nitroprusside and phosphotungstic acid (Burk *J. Biol. Chem.*, 1937, **120**, 63), porphyrindin (Greenstein, *ibid.*, 1939, **128**, 233) and iodide-iodate (Hess *et al.*, *ibid.*, 1943, **151**, 635).

To warrant this assertion, amandin should be treated with reagents able to react with isolated thiols and "sluggish SH groups" (Hellerman *et al.*, *ibid.*, 1943, **147**, 445).

Silver and *p*-chloromercuribenzoate were therefore used as the most powerful selective SH reagents known.

Diluted ferricyanide, under well determined conditions, oxidises only the SH groups in ovalbumin (Anson, *J. Gen. Physiol.*, 1939, **22**, 247). The reaction is stoichiometrical and does not go beyond the disulphide stage. It therefore requires the close proximity of two SH groups. But, the action of ferricyanide on other reducing groups of proteins has never been systematically studied. So, it remained questionable if its specificity for the SH groups extended to proteins other than ovalbumin. It was tentatively applied to amandin.

*Argentometric Amperometric Titrations.*—$Ag(NH_3)_2^+$, a mercaptide-forming reagent, attacks always a maximum number of SH groups. The method of Kolthoff and Harris (*Ind. Eng. Chem. Anal. Ed.*, 1946, **18**, 161) was adapted to semi-micro dosages by using a vibrating electrode according to Rosenberg *et al.* (*Anal. Chem.*, 1950, **22**, 1186). The $HgI_2$ reference cell was replaced by an Ag/AgCl one and a potential of -0.2V was applied to the vibrating Pt electrode. The microammeter was a W. G. Pye Cambridge, with a maximum deflection of 95 mm. per $\mu$A. It was shunted by extra resistances regulated to obtain the desired sensibility.

The titrating solutions (0.005 or 0.001 *M*-$AgNO_3$) were added dropwise with a microburette. An equal volume of buffer solution (0.6 *M*-$NH_4NO_3$ and 0.1 *M*-$NH_3$) at $p_H$ 9.25 was added to the 2-3% protein solutions just before starting the titrations. For each protein a whole set of determinations were performed on volumes of solution ranging from 1 ml. to 20 ml. Subjective influences of one determination on the other, in the ammeter readings and in the drawing of the two lines extrapolation of which furnished the end-point, were thus avoided.

p-*Chloromercuribenzoate.*—*p*-Chloromercuribenzoate (*p*-ClHg-b), another mercaptide-forming reagent, should be more sensitive to steric hindrances. The back-titration of an excess of *p*-ClHg-b by cysteine, in presence of nitroprusside, according to Mac Donnell *et al.* (*Arch. Biochim. Biophys.*, 1951, **32**, 288) was adapted to microquantities, thanks to an "Agla" microsyringe. *p*-ClHg-b was synthesised from *p*-toluenesulphonyl chloride *via* *p*-toluenesulphonate and *p* tolylmercuric chloride according to Whitmore (Gilman & Blatt, "Organic Synthes", 1947, pp. 492, 519, 159). *p*-Chloromercuribenzoic acid (200 g.) was dissolved in 100 ml. of 0.01 *M*-NaOH. The solution was filtered before each set of determinations. The nitroprusside was prepared by crushing together one part of nitroprusside (B. D. H.) and two of $Na_2CO_3$ 'pro analysi'. On a porcelain spotting-plate, several 0.1 ml. *p*-ClHg-b drops were placed. To one half of the drops 0.2 ml. of distilled water was added and to the other half, 0.2 ml. of the 2-3% protein solution. After 1 hour of contact, a few mg. of the nitroprusside were added. The excess *p*-ClHg-b was titrated by a fresh 0 005 *M* cysteine solution until a rosy colour persisted for a few moments under gentle stirring. The difference between the blank and protein titrations expresses the amount of SH groups undergoing reaction. Results were reproducible up to 0.005 $\mu M$ of cysteine.

*Ferricyanide.*—Anson's method (*loc.cit.*) was slightly modified. The ferrocyanide formed was directly determined. At 4000–4200 Å, ferrocyanide absorbs only 4% of what ferricyanide does. A standard curve for ferro-ferricyanide mixtures in different proportions was established under the conditions of the actual SH determinations, with a spectrophotometer (Beckman Du) at 4100 Å and 4200 Å and 25°. A linear relation between ferricyanide concentration and extinction was observed for concentrations from $2 \times 10^{-5}$ *M* to $2 \times 10^{-4}$ *M*. Determinations were made as follows: 4 ml. of a 2-3% protein solution and 4 ml. distilled water were introduced into separate 100 ml. volumetric flasks. *M*/6-Neutral phosphate buffer (1 ml.) and a 0.004*M* ferricyanide solution (5 ml.) were added to both. The reaction was allowed to proceed for 15 minutes at 25°. Water was then added up to the mark, and 10 ml. was pipetted into a test-tube and 1 ml. of a 30% trichloroacetic acid solution added. The precipitated protein was filtered off and the extinction of the filtrate measured at 25°. By reading on the standard curve, the quantity of ferrocyanide corresponding to the difference between the extinctions of protein solution and blank, one can eventually know the number of SH groups involved in the reaction. Readings were repeated at 4100 Å and 4200 Å and precipitations made several times for each determination.

*Proteins.*—All concentration determinations were carried out by semimicro Kjeldhal. Protein solutions were prepared from freeze-dried preparations and stored in the cold room under toluene for never more than 20 days. Denaturation was carried out as follows: To 10 ml. of a 3% protein solution in a 20 ml. volumetric flask 9 g. of urea was added. Distilled water was then added slowly to dissolve the urea and bring the solution to the mark (7.5 *M* in urea). The solutions were left for 1 hour at 25° before titration, unless the time of contact was otherwise specified.

To compare the literature data with ours, the percentages of cysteine reported were eventually converted into SH/M on the basis of the M. W. adopted by us.

## EXPERIMENTAL

*Preparation.*—We are indebted to Prof. P. Putzeys for the amandin, prepared by an unpublished method. The ground almonds were defatted by a 10% NaCl—0.1% $NaHCO_3$ solution. After Büchner filtration, the extract was clarified on kieselguhr and $CaCl_2$. The solution was saturated with $MgSO_4$ and the precipitate discarded. By adding an equal volume of a saturated $(NH_4)_2SO_4$ solution the amandin was precipitated. It was dissolved in the solvent and reprecipitated several times. It was finally dialysed against 0.3*M*-$NH_4NO_3$. As few or no SH groups were expected, 5-7% amandin solutions were used. Dilution with the ammonium buffer solution before the silver amperometric titrations was replaced by the addition of a few drops of $NH_3$ (3*M*) to the 0.3 *M*-$NH_4NO_3$ solution so as to bring it to $p_H$ 9.25.

The M.W. was assumed to be 385,000 (Moody, Thesis, Wisconsin Univ., 1944) and the N%, 18.65.

TABLE I

| | $Ag(NH_3)_2^+$ | | | *p*-ChloroHg benzoate. | | | $Fe(CN)_6^{3-}$ | |
|---|---|---|---|---|---|---|---|---|
| | % Cyst. | SH/M. | No. of determ. | % Cyst | SH/M. | No of determ. | Reduced $Fe^{3+}$/M. | No. of determ. |
| Native protein. | | | | | | | | |
| | 0.036 | 1.2 | 9 | 0.04 | 1.3 | 6 | 5 | 4 |
| Protein denatured in urea (7.5*M*) | | | | | | | | |
| Time of contact. | | | | | | | | |
| ½ hr. | 0.027 | 0.9 | 2 | | | | | |
| 24 | 0.015 | 0.5 | 2 | 0.036 | 1.2 | 6 | 5 | 2 |
| 96 | 0.006 | 0.2 | 2 | | | | | |

Five molecules of ferricyanide are reduced per molecule of amandin, while silver and *p*-ClHg-b react with about 1 SH/M. It is most unlikely that ferricyanide would affect five times more SH groups than the strongest reagents. Moreover, other equally effective oxidising agents failed to detect any free SH group in amandin. Most probably ferricyanide oxidises other groups (tyrosine, tryptophane etc.) and thus cannot be considered as a universally specific reagent for proteinic thiols.

Silver and *p*-ClHg-b titrations show the presence of a little more than one "sluggish SH group" per molecule of amandin. This group is probably responsible for the qualitative positive reaction with nitroprusside and phosphotungstic acid after denaturation by urea, mentioned by Buik (*loc.cit.*). He also noticed that amandin in 6.6*M* urea solution had split into sub-units of M.W. 30,000. The detection of a single "sluggish SH group"/M, would imply that these sub-units are not all perfectly identical.

Amandin seems to be a heterogeneous protein (Barré, *Bull. soc. chim. Biol.*, 1953, **35**, 899, 917). SH determinations should therefore be made on each fraction to detect the possible existence of a "mercaptamandin". Hess *et al.* (*loc.cit.*) found no cysteine

but 28 cystine/M in amandin, hydrolysed by 20% HCl and 6*N*-$H_2SO_4$. An inference from such analysis to the cysteine present in the native molecule, however, remains doubtful.

The slow decrease in the number of SH groups detected by Ag, observed with longer times of contact between amandin and urea, is probably due to a spontaneous oxidation by air and the formation of an intermolecular disulphide bond between two sub-units of amandin.

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# THE REACTIVITY OF SULFHYDRYL GROUPS IN PROTEINS. PART II. LYSOZYME AND OVALBUMIN

By R. Lontie and G. Beckers

Various types of specific thiol reagents were allowed to react on lysozyme and ovalbumin, both native and denatured. An attempt has been made to interpret the differences of reactivity observed in the light of the Pauling-Corey structural theories. The hypothesis of intramolecular hydrogen bonds, involving cysteinic thiol and peptidic carbonyl groups, is brought forward to explain certain differences of reactivity of native ovalbumin.

Since the work of Hellerman *et al.* on urease (*J. Biol. Chem.*, 1943, **147**, 443), the distinction between "freely reacting", sluggish" and "masked" SH groups among the sulfhydryl groups of a protein has become classical. The first reacts with all the thiol reagents, the second with the stronger reagents only, while the masked groups become accessible to reagents only after denaturation.

Much experimental data are already available on this subject, showing similar differences of reactivity in other proteins. Several interpretations have also been proposed, but none are fully satisfactory. Still, before attempting to explain these differences, it seems that a doubt concerning the validity of comparison of the data is to be cleared. SH reactions are deeply influenced by the conditions of the reaction. Most of the authors, however, worked with a single reagent on one or a few proteins. Too often they prepared or denatured their proteins according to different methods. The determinations were performed under different conditions of concentration, temperature, $p_H$, ionic strength, time etc., and in quite a few papers, all these conditions were not even specified. Hence, the ambiguity of a comparison of such data may be ascribed to the discrepancies arising out of the differences in the conditions of operation. To dismiss such a doubt, a few critically chosen methods were applied under standard conditions to one and the same solution of each of a few proteins, both in their native state and after denaturation in a 7.5*M*-urea solution. Silver, *p*-chloromercuribenzoate and ferricyanide determinations were conducted according to the methods described in Part I (this *issue*, p. 285).

*Iodoacetic Acid and Iodoacetamide.*—Alkylating reagents are the mildest among the three types of commonly used SH reagents. Iodoacetamide and iodoacetic acid were selected. Rosner's method (*J. Biol. Chem.*, 1940, **132**, 657) was followed with a few modifications and the replacement of the colorimeter by a spectrophotometer. Under our conditions of work, the alkylating reagents react also with methionine, but more slowly (Hermans, Thesis, Leuven, 1951). The very slow reaction with the amino groups is important owing to their number (Michaelis and Schubert, *J. Biol. Chem.*, 1934, **106**, 331). A rise of temperature or $p_H$ increases considerably the velocity of the reactions. These remarks, and more could be made, show the importance of a perfect standardisation of the methods to ensure a significant comparison of data.

The iodoacetamide was synthesised by Anson's method (*J. Gen. Physiol.*, 1939, **22**, 247) and the monoiodoacetic acid, according to Abderhalden (*Ber.*, 1908, **41**, 2852). The starch solution was prepared according to Gross (*Anal. Chem.*, 1948, **20**, 900). The iodine solution in presence of starch shows, under our conditions of operation, a maximum absorption at 5800Å, increased by 25% through dilution with an equal volume of a saturated $(NH_4)_2SO_4$ solution. But for minute concentrations, the relation between extinction and iodine concentration is perfectly linear.

The determinations were conducted as follows: 15 ml. of a 3% protein solution and 15 ml. of distilled water were introduced in separate glass-stoppered flasks; 15 ml. of the 1% alkylating reagent solution, buffered at neutrality, was added to both. All solutions were prestabilised at 25°. The flasks were placed in a thermostat and, at determined times, 4 ml. of the reacting mixture was pipetted into centrifugation tubes, where the reaction was abruptly stopped by addition of 3 ml. of a 3.3% sulphosalicylic acid solution. The precipitated protein was centrifuged and the supernatant liquid filtered into a 20 ml. volumetric flask. The washing sulphosalicylic solution was added up to the mark. If the amount of $I^-$ formed was expected to be small, 0.8 ml. of a 0.002*M*-KI solution was pre-added to the blank and protein solutions so as to reach the threshold of linearity in the extinction curves. The solutions (5 ml.) were pipetted into small Erlenmeyer flasks and 5 ml. of a saturated solution of $(NH_4)_2SO_4$, 2 ml. starch solution and 3 drops of perhydrol were added. When a large amount of $I^-$ was expected to be liberated, a convenient dilution with the sulphosalicylic acid solution was made in the first stage. Extinctions were measured at 5800Å and at 25° with a Beckman DU. The operations on the filtrate were repeated 2 to 3 times, and the average extinction recorded. The amount of $I^-$ liberated was determined by reading the amount corresponding to the difference between extinctions of blank and protein solutions on pre-established standard curves. These were made from known KI solutions. The quantities of $I^-$ liberated in terms of time of reaction were recorded on a graph. By extrapolating to zero time the "free SH groups" were determined. The slope of the curves provides indications about the other reactions.

## Experimental

### *Lysozyme*

Crystallised lysozyme was prepared according to Alderton *et al.* (*J. Biol. Chem.*, 1945, **157**, 43; 1946, **164**, 1).

Four argentometric titrations were performed with each of the native, urea- and heat-denatured protein solutions. No SH group was detected. This corresponds with the results of the amino-acid analysis which accounts for no cysteine. The presence of a large amount of cystine (8%) confirms the fact that under our conditions of operation, the silver leaves disulphide bonds untouched in proteins. In view of these findidgs, it is difficult to see how the hypothesis of Burk (*J. Biol. Chem.*, 1937, **20**, 63) that the appearance of the "masked SH groups" after denaturation is due to the breaking of disulphide bonds by the denaturing agents, could still be defended.

*Ovalbumin*

Crystallised ovalbumin was prepared according to Sörensen *et al.* (*Compt. rend. Trav. Lab. Carlsberg*, 1917, **12**, 68). The solutions A, B, C and D were prepared directly from egg white and E, from egg white first treated with bentonite to obtain the lysozyme. The M. W. of 45,000 (Bull, *J. Biol. Chem.*, 1941, **137**, 143) and N-content of 15.8% (Lewis *et al.*, *ibid.*, 1950, **186**, 23) were adopted.

TABLE I

*Titrations.*

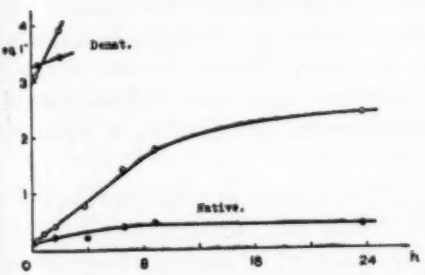
| Soln. | $Ag(NH_3)_2^+$ SH/M. | No dets. | *p*-ClHg-b. SH/M. | No. | $Fe(CN)_6$. SH/M. | No. | $ICH_2COO^-$. SH/M. | $ICH_2CONH_2$. SH/M. |
|---|---|---|---|---|---|---|---|---|
| | | | Native egg albumin | | | | | |
| A | 5.6 | 2 | | | | | | 0 1 |
| B | 4.8 | 3 | 3.2 | 9 | 0 | 3 | 0.1 | |
| C | 5.6 | 3 | | | | | | |
| D | 5.5 | 6 | | | | | | |
| E | 4 4 | 2 | 3.1 | 5 | 1.2 | 3 | | |
| Lit. | " | | 2.3-3.5 | Mc.Do. | 0 My. & An. | | 0 My. & A Ro. | 0 Lar. |
| | | | Egg albumin denatured with 7.5 *M* urea. | | | | | |
| C | 4.1 | 3 | 5.5 | 5 | 1/2hr 2 | 2 | | |
| | | | | | 1hr. 2.7 | 2 | | |
| | | | | | 30hr. 1.8 | 2 | | |
| B | | | | | | | 3 25 | |
| Lit. | | | | | 3.6 My. | | 3.1-3.4 Ro. | 3 |

No: for number of separate determinations; average result recorded.
Mac Donnell (*loc. cit.*); Mirsky & Anson (*J. Gen. Physiol.*, 1936, **19**, 451); Rosner (*loc. cit.*); Larson & Jenness (*J. Amer. Chem. Soc.*, 1952, **74**, 3090); Mirsky (*J. Gen. Physiol.*, 1941, **24**, 709).

Alkylating reagents do not afford an immediate reaction with the SH groups of native egg albumin. If the protein, however, is kept sufficiently long in contact with iodoacetamide, and denatured after the iodoacetamide has been washed away, the SH reagents react with 2 SH/mol. less than they normally do. A slow reaction with iodoacetamide before denaturation was accordingly surmised (Anson, *loc. cit.*, 1940; Larson, *loc. cit.*). The slope of the curves (Fig. 1) directly confirms this hypothesis: for the first 10 hours the slope of the iodoacetamide reaction with the native protein is steep while that of iodoacetate is practically non-existant. We know that SH reactions are swifter with iodoacetamide than with iodoacetate (Dickens, *Biochem. J.*, 1933, **27**, 1141), while with most amines it is the velocity of the iodoacetate reaction which is greater (Michaelis and Schubert, *loc. cit.*). It may then be safely concluded that the two iodine equivalents liberated within the first 10 hours of the reaction are due to slow iodoacetamide-SH reaction.

FIG. 1

*I⁻ equiv. liberated per mol. of protein in terms of time.*




Curves marked with black dots and circles represent respectively the reaction of iodoacetate and iodoacetamide with native and urea-denatured ovalbumin.

## Discussion

Ovalbumin has been the most extensively studied protein concerning SH reactions. Our conclusions are based on the literature as well as on our own results. References to papers reviewed by Anson (*Adv. Prot. Chem.*, 1945, **2**, 361), Neurath *et al.* (*Chem. Rev.*, 1944, **34**, 157), Herriott (*Adv. Prot. Chem.*, 1947, **3**, 170) and Barron (*Adv. Enzym.*, 1951, **11**, 201) have been omitted. Four groups of phenomena call for interpretation :

1. *The reactivity of "masked* SH *groups" after denaturation.*—From the amino-acid analysis, 5-6 cysteine radicals per mol. could be expected. Silver reacts with 5.5SH/mol. in the native protein, *i.e.*, probably all the SH groups present. Ovalbumin is in fact a mixture of two closely similar proteins in proportions varying according to the sample. They possess different electrophoretic mobilities at certain $p_H$, but have not been fractionated (Longsworth *et al.*, *J. Amer. Chem. Soc.*, 1940, **62**, 2580). One component possesses probably 5 cysteine/mol. and the other 6. Other reagents than silver attack either some of the groups or none. After denaturation in a 7.5 *M*-urea solution, however, all "masked" groups react with *p*-ClHg-b, 2-3 groups with ferricyanide and other oxidising agents, 3 with the alkylating agents. Many similar observations are reported for other reagents and denaturating agents. A comprehensive explanation of these phenomena is required, as groups other than the thiols are also masked in native proteins.

The picture of denaturation in the light of the Pauling-Corey structural theories seems to account satisfactorily for the increase of reactive groups.

Native ovalbumin shows the α-keratin X-ray diagram, related by Pauling to twisted α-helixes. The molecule is formed of a single polypeptide of about 400 amino-acids. It appears to be a cylinder or an ellipsoid, 90-100Å high with a diameter of 26-32Å. The helix is thus plaited, to form the "7 strand coiled coil" suggested by Pauling or some similar structure. Physicochemical studies have led to represent the molecule of denatured ovalbumin in solution as a rigid rod of 600Å in length. It indicates that it is a simple helix with 1.5Å per radical. Moreover, the X-ray diagram remains characteristic of the α-helix. Finally, by the action of a drastic agent or of some mechanical force, the denatured molecule acquires the β-keratin structure, corresponding to a plaited sheet configuration.

Thus, the denaturation of ovalbumin consists in the rupture of weak intrapeptidic bonds, resulting in a progressive unfolding of the "coiled coil", accompanied eventually by local uncoiling of the helix itself. In partially denatured ovalbumin, there seems to be an equilibrium mixture between native and denatured protein. Practically all of the latter exist under a single form corresponding to the actual chemical environment (Strachitskii and Plotnikova, *Ukrain Biokhim. Zhur.*, 1948, **20**, 187). Denatured molecules tend to aggregate by the formation of disulphide intermolecular bonds (Halwer, *J. Amer. Chem. Soc.*, 1954, **76**, 183). The reactivity of SH groups increases with this unfolding, as would be expected. Where closely intertwisted plaited helixes predominate, the side chains will be involved in bonds of different types, either with nearby radicals or with the very imino and carbonyl groups of the chains, or they will be coiled

up in the "holes" of the helixes. The rigid structure stabilises the otherwise weak bonds; it maintains also a fixed constellation of radicals surrounding the non-peripheral reactive groups, and thus renders them inaccessible to certain reagents. With the unfolding and eventual local uncoiling of the helixes, however, many electrostatic and steric hindrances are removed, stabilised intrapeptidic bonds are broken, and the side radicals regain a large degree of freedom that enables them to react more easily.

Some parallel observations are enlightening. Bull (*J. Biol. Chem.*, 1940, **133**, 39) showed that the viscosity of an ovalbumin solution increased notably with denaturation by heat, more so in presence of urea. Connecting viscosity with the assymetry of the molecule, he calculated that the axial ratio passed from 3.9 to 7.4 and 9.2 respectively. These ratios manifest a progressive unfolding to which may be connected an increased SH reactivity (e.g., Mirsky, *J. Gen. Physiol.*, 1941, **24**, 709 ; by ferricyanide : 0; 2; 3.6 SH/mol. respectively).

2. *Spontaneous oxidation by air of some thiols of denatured ovalbumin.*—Fewer SH groups (4.1/5.6) were detected by silver after urea-denaturation. Benesch (*Arch. Biochem.*, 1948, **19**, 35) made similar observations on ovalbumin denatured with guanidine, alcohol and duponol. The spontaneous oxidation of thiols to disulphides by air is known. The reaction velocity increases with the temperature, $p_H$ and the presence of traces of metals (Barron, *Biochem. J.*, 1947, **41**, 62). We may thus expect oxidation to occur in denatured ovalbumin whenever two SH groups come sufficiently close together. Intermolecular bonds are surely created that way and aggregates formed (Halwer, *loc. cit.*). We attribute the decrease of the silver reaction to such oxidations, occurring in the time between the preparation of the buffered ammoniacal solution and the end of the titration (¼ to ½ hour). The greater freedom enjoyed by the unfolded molecules, as denaturation proceeds, allows SH groups to come in such closeness as to permit the formation of disulphide bonds. These reactions will follow the general rules of thiol oxidation. We may thus expect a much swifter oxidation by air at the $p_H$ 9.25 of the silver titration than at the neutral $p_H$ of the other methods (so *p* ClHg-b reacts with all the SH groups [5.5] after denaturation).

The percentage of spontaneously oxidised thiols is bound to increase also with longer times of contact of the protein with the denaturating agent. This would explain the ferricyanide titrations. There is at first a slight increase of SH groups titrated (2 to 2.7 SH/mol.) with longer time of contact with urea (½ to 1 hr.). The unfolding is probably still progressing. This increase is reversed (2.7 to 1.8 SH/mol.) by spontaneous oxidation, if the time of contact is unduly prolonged (1 to 30 hr.). Rosner (*loc. cit.*) observed a similar decrease in his titrations of denatured ovalbumin using iodoacetate. So did Hopkins with nitroprusside (*Nature*, 1933, **126**, 328). These phenomena show the importance of a perfect standardisation of the methods. It would be safer to conduct the denaturation at a low $p_H$ (5.5) and neutralise the solutions just before the titrations.

3. *The different reactivity of the thiols of native ovalbumin with different specific reagents.*—The responsibility for the differential reactivity of the SH groups of native ovalbumin, under identical conditions, with the different specific reagents may be attributed to three main causes :

(*a*). Mild oxidising agents affect no thiols in native ovalbumin (ferricyanide, porphyrindine, aresenoxide, 2:6-dichlorophenol-indophenol, etc.). Thiols, in close proximity with each other only, can be oxidised into disulphides. There are presumably no such thiols in native ovalbumin.

Data of titrations by oxidising agents must be carefully handled. The specificity of their SH reaction is always doubtful.

(*b*). Thiols could be rendered inaccessible to certain reagents due to steric or electrostatic hindrances brought about by neighbouring chains. The inhibitory effect of valine in the polarographic reduction of phenacetyl-L- cysteinyl-D- valine mercaptides, and its disappearance in urea solution are suggestive in that line (Benesch, *J. Amer. Chem. Soc.*, 1953, **75**, 4367). For lack of knowledge regarding the amino-acid sequence, however, such interpretations of a differential reactivity in proteins remain questionable.

We must notice the complete titration of ovalbumin SH groups by the small silver ion (5.5 SH/mol.) and their incomplete titration by equally strong but cumbersome *p*-ClHg-b (3.1 SH/mol.), while the cysteyl-valine sequence has been found to be one of the important dipeptides in ovalbumin hydrolysates (Flavin, *Nature*, 1954, **173**, 214).

(*c*). Proteinic thiols seem capable of forming hydrogen bonds. Further work on the subject is being carried on and details will be published later. Some points may be mentioned here. The possibility of hydrogen bonds involving an SH group as proton donor is now-a-days established. The strength of the bond is proportional to the ionic character of the thiol. Accordingly, alkalymercaptans form only weak bonds (specially Gordy and Stanford, *J. Amer. Chem. Soc.*, 1940, **62**, 497). Yet, from the general conditions of hydrogen-bond formation, it appears that favourable atomic distances and bond angles could stabilise an otherwise weak bond. This is the case in many instances of chelation, involving Cl,Br or certain C atoms. In a molecular modal of an α-helix, besides other possibilities, favourable conditions seem to exist for the formation of hydrogen bond between the sulphur of a cysteinyl side chain and the oxygen of its carboxy group. Bond distances and angles are such as to allow the formation of a six-membered ring (including the H atom). The co-planarity of the carbonyl group and α-carbon is imposed by resonance in the peptide linkage. The electronegativity of the oxygen is greatly enhanced for the same reason. Inferring from analogous structures and X-ray data, we expect the structure with a co-planer β-carbon to be favoured. Only the SH group would enjoy a large freedom of rotation. Keeping normal bond angles and distances, a vector from the S atom to the O atom could be be deflected only by a few degrees from the SH direction, while the distance between the H and O atoms would be of about 1.5Å, if the $C_\beta$—S bond forms an angle of about 50° with the CO—$C_\alpha$—$C_\beta$ plane. The increase in ionic character noted for the thiol of glutathione as compared with that of cysteine (Cecil, *Biochem. J.*, 1950, **47**, 572) could find here its explanation. On the other hand, we have noticed that the reactions of cysteine with silver and *p*-ClHg-b in electron-donor solutions are quantitative. The latter, however, affect the reactions with ferricyanide and easily reduce by one half the reactions of the alkylating reagents. The effects observed were found to be a function of the basicity and concentration of electron-donor (Beckers, Thesis, Louvain, 1953). Benesch (*loc. cit.*, 1953) supplies an interesting counter-proof. In the

presence of urea, the nitroprusside reaction of cysteine is increased by 50%, that of three cysteinic tripeptides, by 100%, whereas the reactions with $Na_2S$ and ethylmercaptan are unaffected by the presence of urea.

To sum up: hydrogen bonds involving the thiols are likely to occur in proteins, and such bonds seem to affect the reactions of thiols with mild reagents and not those with stronger ones. Hence, new possibilities of discrimination in the action of specific reagents.

We are inclined to think that the three thiols of native ovalbumin which afford no reaction with iodoacetate and other reagents, a very slow and partial reaction with iodoacetamide and which react normally both with silver and *p*-ClHg-b, fall under this heading. These may even be the groups that remain "sluggish" after denaturation, while the other two groups become accessible by unfolding of the molecule.

(*d*). *Discrepancies in the titrations of an apparently identical protein with the same reagent.*—We notice appreciable differences between the titrations of the E solution of ovalbumin and the other solutions. The silver reagent titrates only 4.4SH/mol. instead of 5.5 SH, and by ferricyanide we detect 1.2 SH/mol. instead of 0.

This is the place to stress the importance of a perfect standardisation in the mode of preparation of the portein. When a protein is a mixture of two components, we can always expect that the proportions may vary according to the mode of preparation. Conditions under which oxidation of SH groups by air could be enhanced, such as high $p_H$, contact with worn brass agitator etc., should be avoided. The same must be said of procedures that could provoke a partial denaturation.

The E solution was prepared from egg white first treated with bentonite to extract the lysozyme. The suspension was homogenised in a Waring blender. Likely, the mechanical agitation provoked a beginning of denaturation (cf. Wu and Ling, *Chinese J. Physiol.*, 1927, **1**, 407) that manifested itself by the availabilty of one "masked" SH group . It would be the one titrated directly by ferricyanide and oxidised by air in the solution buffered at $p_H$ 9.25 for the silver titration.

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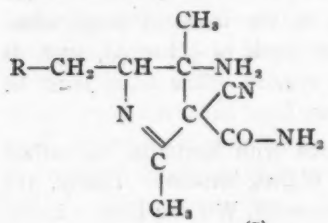
# ON THE SYNTHESIS OF $\Delta^1$- PYRROLINE DERIVATIVES. PART II

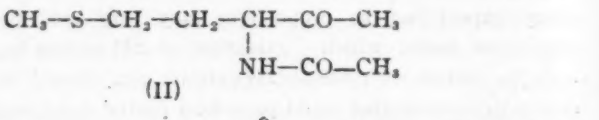
By T. N. Ghosh and Saktipada Dutta

The reaction of an α-acetamidoketone with ethyl cyanoacetate or its amide in presence of ammonia to furnish a $\Delta^1$-pyrroline derivative, as reported previously (Ghosh and Dutta, this *Journal*, 1955, **32**, 719), has now been extended with several other α-acetamidoketones.

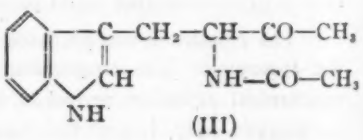
In a previous communication (Ghosh and Dutta, *loc. cit.*) it has been shown that an α-acetamidoketone, when allowed to react with ethyl cyanoacetate or cyanoacetamide in presence of ammonia, furnishes a compound, possibly represented by the amino-pyrroline structure (*I*). The mechanism suggested for its production is based on the initial formation of a carbinol-amine which then reacts with cyanoacetamide (cf. Robinson, *J. Chem. Soc.*, 1917, **111**, 876) and the resulting intermediate readily cyclises, under the experimental condition, to form (I), the constitution of which is supported on the ground that the compound shows the presence of a free amino, a cyano and a carboxamido group.



(I)



(II)



(III)

In order to study the general applicability of the above reaction, it has been considered desirable to prepare various α-acetamidoketones and condense them with ethyl cyanoacetate or cyanoacetamide in presence of ammonia. It is now found that (α-acetamido-β-*o*-chlorophenyl)-ethylmethyl ketone (Ghosh and Dutta, this *Journal*, 1955, **32**, 755) furnishes the amino-pyrroline (I, R = *o*-Cl − $C_6H_4$), when treated with ethyl cyanoacetate in presence of ammonia. (γ-Methylmercapto-α-acetamido)-propylmethyl ketone (II), obtained by subjecting methionine to the Dakin and West reaction (*J. Biol. Chem.*, 1928, **78**, 91, 745, 757) with acetic anhydride in presence of pyridine, has also been found to afford (I, R = $CH_3-S-CH_2$) under similar conditions. However, [α-acetamido-β (3-indole)]-ethylmethyl ketone (III), obtained as a heavy, viscous, brownish liquid by subjecting tryptophane to the Dakin and West reaction and characterised as 2 : 4-dinitrophenylhydrazone, yielded, when treated with ethyl cyanoacetate in presence of ammonia, a liquid which did not form any picrate or acetyl derivative and could not be characterised.

It is significant to observe in this connection that no basic compound could be obtained by treating an α-acetamidoketone, under identical conditions, with cyanoacetamide in presence of a trace of piperidine, instead of ammonia.

## EXPERIMENTAL

*2:4-Dimethyl-3-cyano-3-carboxamido-4-amino-5-o-chlorobenzyl-$\Delta^1$-pyrroline* (I, R = *o*-Cl-$C_6H_4$).—To anhydrous ethanol (50 c.c.), saturated at 0° with dry ammonia gas, (α-acetamido-β-*o*-chlorophenyl)-ethylmethyl ketone (10 g.) and ethyl cyanoacetate (10 g.) were added and the mixture was kept in the refrigerator in a well-closed bottle for 96 hours. Removal of ethanol left a solid which was purified by solution in cold dilute HCl and precipitation by a cold dilute NaOH solution. The solid was finally crystallised from ethanol in colorless needles (6 g.), m.p. 209-10°. (Found: C, 58.78; H, 5.41; N, 18.12. $C_{15}H_{17}ON_4Cl$ requires C, 59.11; H, 5.58; N, 18.39 per cent).

The *picrate* was crystallised from ethanol in yellow needles, m.p. 198-200°(decomp.). (Found: N, 18.23. $C_{15}H_{17}ON_4Cl.C_6H_2O_7N_3$ requires N, 18.37 per cent).

The 4-*acetamido* derivative was obtained by treatment with acetic anhydride in presence of acetic acid, purified by trituration with cold dilute HCl and it was crystallised from glacial acetic acid-ethanol mixture (1:1) in fine colorless needles, m.p. 271-72°. (Found: N, 15.78. $C_{17}H_{19}O_2N_4Cl$ requires N, 16.16 per cent).

The *4-allylthiocarbamide* derivative was obtained by refluxing for about 6 hours an ethanolic solution of (I, R = *o*-Cl-$C_6H_4$) and a slight excess of allyl *iso*thiocyanate. The solid obtained by removal of ethanol was triturated first with ether and then with cold dilute HCl and was finally crystallised from ethanol in colorless needles, m.p. 183-84°. (Found: N, 17.12; S, 7.56. $C_{19}H_{22}ON_5SCl$ requires N, 17.34; S, 7.93 per cent).

*(γ-Methylmercapto-α-acetamido)-propylmethyl Ketone* (II).—A mixture of *dl*-methionine (10 g), acetic anhydride (42 c. c.) and pyridine (20 c.c.) was heated under reflux on a water-bath for 6 hours. Pyridine and the excess of acetic anhydride were removed by steam-distillation and the mixture was cooled in ice and then extracted with ether. The ethereal extract was carefully washed with cold aqueous sodium bicarbonate solution (5%) and then with water and was finally dried over anhydrous sodium sulphate. Removal of the ether left a heavy, viscous liquid (6.5 g.) which could not be distilled without decomposition, even under reduced pressure. The ketone was characterised as *2:4-dinitrophenylhydrazone*, which crystallised from ethanol in fine, yellow needles, m.p. 189 90°. (Found: N, 18.85. $C_{14}H_{19}O_5N_5S$ requires N, 18.97 per cent).

*2:4-Dimethyl-3-cyano-3-carboxamido-4-amino-5-(β-methylmercapto)-ethyl-$\Delta^1$-pyrroline* (I, R = $CH_3-S-CH_2$).—Using (γ-methylmercapto-α-acetamido)-propylmethyl ketone and proceeding as in the case of (I, R = *o*-Cl-$C_6H_4$), (I, R = $CH_3$-S-$CH_2$) was obtained and crystallised from ethanol in fine, pinkish needles, m.p. 162°. (Found: N, 21.87; S, 12.12. $C_{11}H_{18}ON_4S$ requires N, 22.05; S, 12.59 per cent).

The *picrate* was obtained from ethanolic solution as yellow needles, m.p. 167-68°. (Found: N, 20.15. $C_{11}H_{18}ON_4S.C_6H_3O_7N_3$ requires N, 20.29 per cent).

The *4-acetamido derivative* was obtained by employing a mixture of acetic anhydride and acetic acid and was crystallised from a mixture of ethanol-acetic acid mixture (1:1) in colorless needles, m.p. 220°. (Found: N, 18.73. $C_{13}H_{20}O_2N_4S$ requires N, 18.92 per cent).

[*α-Acetamido-β-(3-indole)*]*-ethylmethyl Ketone* (III).—A mixture of *dl*-tryptophane (12 g.), acetic anhydride (45 c.c.) and pyridine (32 c.c.) was heated for about 6 hours under reflux on the water-bath in a flask protected from moisture. After steam-distillation the mixture was cooled and extracted with ethyl acetate. The ethyl acetate extract was successively washed with water, cold aqueous sodium bicarbonate solution (5%) and then with water and was dried over anhydrous sodium sulphate. Removal of ethyl acetate left a heavy, viscous, brownish liquid (11.5 g.), which could not be distilled without decomposition, even under reduced pressure. It was characterised as 2:4-*dinitrophenylhydrazone*, crystallised from ethanol in brown needles, m.p. 265-66° (decomp.). (Found : N, 19.64. $C_{20}H_{20}O_5N_6$ requires N, 19.81 per cent).

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# STUDIES ON METAL FERROCYANIDES. PART II. VOLUMETRIC DETERMINATION OF TRIVALENT CERIUM BY FERROCYANIDE

By G. S. Deshmukh and M. Venugopalan

Precipitation of $CeKFe(CN)_6$ from a solution of cerous chloride with potassium ferrocyanide of varying concentrations has been carried out for the determination of $Ce^{III}$. The excess ferrocyanide is titrated with standard ceric sulphate and from the changed ferrocyanide the amount of $Ce^{III}$ calculated. This new volumetric procedure has been found to provide sufficiently accurate results under the conditions specified.

The formation of cerium-ferrocyanide complexes of the composition $CeKFe(CN)_6$ in the gravimetric determination of trivalent cerium, involving the precipitation of $CeKFe(CN)_6,4H_2O$ and subsequent weighing of the precipitate, has been reported by Spacu and others (Spacu, *Z. anal. Chem.*, 1936, **104**, 119 ; Atanasiu, *ibid.*, 1936, **105**, 422 ; Schenijakin and Volkova, *J. Gen. Chem. USSR.*, 1937, **17**, 1328, 1332). In the present paper the above procedure of Spacu has been extended to the determination of $Ce^{III}$ by a volumetric method involving the formation of $CeKFe(CN)_6$ from a solution of cerous chloride, with a known excess of ferrocyanide and indirect estimation of the excess ferrocyanide by standard ceric sulphate.

## Experimental

A stock solution of $CeCl_3$ was prepared by dissolving a pure Merck sample of cerous chloride in water. The weight of Ce in this solution was determined by precipitation as cerous oxalate, subsequent ignition to ceric oxide and weighing as $CeO_2$. Potassium ferrocyanide solutions of a pure Merck sample of different concentrations, varying from 1 to 4%, were prepared and the exact concentration determined by titrations with standard ceric sulphate.

A known quantity of cerous chloride solution was pipetted into a beaker to which was added a known excess of $K_4Fe(CN)_6$ solution, and kept aside overnight with frequent shaking. Arrangement for filtration was made using filter cones of Whatman No. 50, provided with suction arrangements. The excess of ferrocyanide was filtered off slowly, then the precipitate was transferred and washed well with water till all ferrocyanide was washed off in the filtrate. To the above filtrate 20 c.c. of 4-5*N*-$H_2SO_4$ was added, and a drop of phenanthroline-ferrous complex as indicator, and titrated against standard ceric sulphate solution to the end-point. A preliminary titration of a known quantity of ferrocyanide was carried out against standard ceric sulphate. From the titre values, the amount of ferrocyanide reacting was calculated.

Different concentrations of the ferrocyanide varying from 1 to 4% solution were tried and the accuracy of the method for different concentrations of cerium was worked out. For the titration relationship the precipitate appears to have the composition $CeKFe(CN)_6$ for the different concentrations of ferrocyanide studied. Typical sets of results are recorded in Table I.

TABLE I

| Vol. of $CeCl_3$. | Conc. of $K_4Fe(CN)_6$. | Wt. of Ce by ferrocyanide. | Wt. of Ce by cerous oxalate. | Diff. |
|---|---|---|---|---|
| 5 c.c. | 25 c.c. of 1% soln. | 0.01503 g. | 0.01498 g. | 0.00005 |
| ,, | ,, 2 | ,, | ,, | ,, |
| ,, | ,, 4 | ,, | ,, | ,, |
| 10 | 25 of 1 | 0.03006 | 0.02996 | 0.00010 |
| ,, | ,, ,, 2 | ,, | ,, | ,, |
| ,, | ,, ,, 4 | ,, | ,, | ,, |
| 12 | 25 of 2 | 0.03582 | 0.03595 | 0.00013 |
| ,, | ,, ,, 4 | ,, | ,, | ,, |
| 14 | 25 of 2 | 0.04163 | 0.04194 | 0.00031 |
| ,, | ,, ,, 4 | ,, | ,, | ,, |
| 15 | 25 of 1 | 0.04513 | 0.04494 | 0.00019 |
| ,, | ,, ,, 2 | ,, | ,, | ,, |
| 20 | 50 of 1 | 0.06017 | 0.05992 | 0.00025 |
| ,, | 25 of 2 | 0.05954 | ,, | 0.00038 |
| ,, | 25 of 4 | 0.05954 | ,, | 0.00038 |
| 25 | 50 of 1 | 0.07469 | 0.07498 | 0.00029 |
| ,, | 25 of 2 | 0.07406 | ,, | 0.00092 |
| ,, | 25 of 4 | 0.07406 | ,, | 0.00092 |
| 30 | 50 of 1 | 0.08828 | 0.08988 | 0.00160 |
| ,, | 25 of 2 | ,, | ,, | ,, |
| ,, | 25 of 4 | ,, | ,, | ,, |

The complex formation observed in this case is essentially supported by the conclusions of Spacu and others (*loc. cit.*).

In addition to the accuracy of the method, the importance of a volumetric method of determination of $Ce^{III}$ with ferrocyanide, involving indirect titration of the ferrocyanide with ceric sulphate, *i.e.* $Ce^{IV}$, is of high value. Again the volumetric procedure involves less complications and the error is less as compared to gravimetric procedures.

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# GIBLING'S CORRECTIONS. PART II

By A. M. Talati

Group parachor values for the groups $(C)CH_2Cl$, $(C)_2CHCl$ and $(C)_3CCl$ have been evaluated from the parachor values of aliphatic monochlorides. The differences between the parachor values of $(C)CH_2Cl$, $(C)_2CHCl$ and $(C)_3CCl$ are similar to those found by Gibling in case of $(C)CH_3$, $(C)_2CH_2$ and $(C)_3CH$ respectively. The results are discussed as regards the interference correction for C—C—Cl.

In continuation of the previous communications (this *Journal*, 1955, **32**, 471, 799) the interference correction for C—C—Cl is derived from the parachor values of aliphatic monochlorides in the present paper. Gibling (*J. Chem. Soc.*, 1941, 299, 306 ; 1944, 384) obtained the interference correction values for C—C—C, C—C—O and C—C—S as 2.2, 2.6 and 2.7 respectively and assumed the interference correction for C—C—N as 2.4 (*J. Chem. Soc.*, 1943, 149). Although Gibling (*ibid.*, 1943, 151) has calculated the group parachor values for the groups $(C)CH_2Cl$, $(C)CH_2Br$ and $(C)CH_2I$, no mention has been made by him as regards the interference corrections for C—C—X, where X may be a halogen atom. In the present paper, group parachor values for the groups $(C)CH_2Cl$, $(C)_2CHCl$ and $(C)_3CCl$ have been derived from the parachor values of ethyl, *iso*propyl and *tert.*-butyl chloride respectively.

The mean of the observed parachor values of ethyl chloride (Mme. Hennant-Roland and Lek, *Bull. Soc. chim. Belg.*, 1931, **40**, 177 ; Vogel, *J. Chem. Soc.*, 1943, 636) may be taken as 151.7. From this the group parachor value for the group $(C)CH_2Cl$ may be calculated as 96.3.

Similarly the mean observed parachor value of *iso*propyl chloride (Vogel, *loc. cit.*) may be taken as 191.6. From this the group parachor value for the group $(C)_2CHCl$ may be derived as 80.9.

Further, the mean of the observed parachor values of *tert.*-butyl chloride (Quayle, Owen and Beavers, *J. Amer. Chem. Soc.*, 1939, **61**, 3107 ; Vogel, *loc. cit.*) is found to be 229.4. This suggests that the group parachor value for the group $(C)_3CCl$ will be 63.3.

These values are recorded in Table I together with those for the groups $(C)CH_3$, $(C)_2CH_2$ and $(C)_3CH$.

Table I

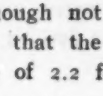
| Group. | [P]. | Diff. | Group. | [P]. | Diff. |
|---|---|---|---|---|---|
| $(C)CH_2Cl$ | 96.3 | ... | $(C)CH_3$ | 55.2 | ... |
| $(C)_2CHCl$ | 80.9 | 15.4 | $(C)_2CH_2$ | 39 8 | 15.4 |
| $(C)_3CCl$ | 63.3 | 17.6 | $(C)_3CH$ | 22.2 | 17.6 |

It is seen from the results that the differences between the parachor values of successive groups show a constant increment of 2.2 in both series. The results indicate that the chlorine atom in aliphatic monochlorides appears to resemble the hydrogen

atom as regards the interference caused by these atoms. The ideal R. P. values for the groups $(C)CH_2Cl$, $(C)_2CHCl$ and $(C)_3CCl$ will be 96.3, 83.1 and 69.9 respectively.

Although Gibling has not derived value of the interference correction for C—C—Cl, following his method of assumption, we may derive it as follows: Since the interference correction for C—C—C, C—C—N and C—C—O are 2.2, 2.4 and 2.6 respectively, the interference correction for C—C—F may be taken as 2.8. Further, the interference correction for C—C—S is 2.7; hence the interference correction for C—C—Cl may be assumed to be 2.9. Using this value and the group parachor value for the group $(C)CH_2Cl$ as 96.3, the group parachor values for the groups $(C)_2CHCl$ and $(C)_3CCl$ may be calculated as 78.7 and 58.9 respectively. These results, however, are not in agreement with the observed values.

The author has shown that the interference corrections for C—C—C, C—C—N and C—C—O have the same value of 2.2. Hence, it would be likely that the interference correction for C—C—F and C—C—Cl may also have the same value of 2.2. But the present results indicate that the interference correction for C—C—Cl is zero. These results indicate the necessity of modification of the concept of interference. According to Gibling (*J. Chem. Soc.*, 1941, 299), the effective volume contribution of a given atom will depend on the nature of the atoms to which it is linked. Interference is bound to occur when atoms, though not directly united, are brought into a close conjunction. On the assumption that the smallest hydrogen atom would cause no interference, he obtained a value of 2.2 for the interference caused by the non-linked atoms $C_1$ and $C_3$ in



It is now seen that the "relatively large" chlorine atom in

also produces no interference with $C_1$. Thus, the variation of the value of the interference correction with the size of the atoms (in the grouping C—C—X) is not so simple as it appears from the values obtained by Gibling.

The author is grateful to Dr. M. D. Avasare, for providing all facilities for the work and for taking keen interest in it.

CHEMISTRY DEPARTMENT,
V. P. MAHAVIDYALAYA,
VALLABHVIDYANAGAR,
VIA ANAND

*Received November 7, 1955.*

* Present address: Petlad College, Petlad (via Anand)

[Jour. Indian Chem. Soc., Vol. 33, No.4, 1956]

# A NOTE ON PARACHOR AND MOLECULAR REFRACTIVITY

By A. M. Talati

Herz (*Z. anorg. Chem.*, 1927, **159**, 316) from the data available for some 51 organic liquids has shown that the quotient of parachor divided by molecular refractivity is a constant, independent of the nature of the liquid. Preliminary investigation showed that the differences in the values of the constant for different compounds were not negligible. The ratio $[P]/[R]$ for different homologous series of the aliphatic compounds of the type RX (where R is an *n*-alkyl group and X = Cl, Br, I, H, OH, SH, or $NH_2$) has been investigated in detail in the present paper (for the values of $[P]$ and $[R]$ vide Vogel *et al.*, *J. Chem. Soc.*, 1940, 171; 1943, 16, 636; 1948, 616, 624, 1814, 1825; Mumford and Phillips, *ibid.*, 1950, 75). The calculated values ($[P]/[R]_{exp}$) are presented in Table I. The results indicate that (*i*) the ratio has a constant value of 8.60 for *n*-alkyl bromides; (*ii*) the ratio has a constant value of 8.50 for *n*-alkyl mercaptans; (*iii*) the value of the ratio is decreasing as the series is ascended in the case of *n*-alkyl chlorides, alcohols and amines and *n*-paraffins; and (*iv*) the value of the ratio is increasing as the series is ascended in the case of *n*-alkyl iodides. It is noted that in the case of those series where the varying values of the ratio are obtained, the values tend to approach a constant value of 8.6 as the series is ascended. Sulphur compounds differ from the rest of the compounds considered here in having a different constant value of 8.50.

TABLE I

(*a*) *n*-Alkyl chlorides.

| *n*-Alkyl group. | $[P]/[R]_{exp.}$ | $[P]/[R]_{der.}$ | Diff. |
|---|---|---|---|
| *n*-Butyl | 9.06 | 9.09 | −0.03 |
| *n*-Hexyl | 8.94 | 8.94 | ±0.00 |
| *n*-Octyl | 8 88 | 8.87 | +0.01 |
| *n*-Decyl | 8.84 | 8.82 | +0 02 |

(*b*) *n*-Alkyl bromides.

| | | | |
|---|---|---|---|
| Ethyl | 8.69 | 8.60 | +0.09 |
| *n*-Butyl | 8.60 | 8.60 | ±0.00 |
| *n*-Hexyl | 8.60 | 8.60 | ±0.00 |
| *n*-Octyl | 8.61 | 8.60 | +0.01 |
| *n*-Decyl | 8.60 | 8.60 | ±0 00 |

(*c*) *n*-Alkyl iodides.

| | | | |
|---|---|---|---|
| Ethyl | 7.71 | 7.68 | +0.03 |
| *n*-Butyl | 7.90 | 7 94 | −0.04 |
| *n*-Hexyl | 8.09 | 8.09 | ±0.00 |
| *n*-Octyl | 8 17 | 8.18 | −0.01 |

(*d*) *n*-Alkyl mercaptans

| *n*-Alkyl group. | $[P]/[R]_{exp.}$ | $[P]/[R]_{der.}$ | Diff. |
|---|---|---|---|
| Ethyl | 8.51 | 8.50 | +0.01 |
| *n*-Butyl | 8.53 | 8.50 | +0.03 |
| *n*-Hexyl | 8.54 | 8.50 | +0.04 |
| *n*-Octyl | 8.51 | 8.50 | +0.01 |

(*e*) *n*-Alkyl alcohols.

| | | | |
|---|---|---|---|
| Ethyl | 9.83 | 9.81 | +0.02 |
| *n*-Butyl | 9.27 | 9.29 | −0.02 |
| *n*-Hexyl | 9.10 | 9.08 | +0.02 |

(*f*) *n*-Alkyl amines.

| | | | |
|---|---|---|---|
| *n*-Butyl | 9.12 | 9.12 | ±0 00 |
| *n*-Hexyl | 8.97 | 8.96 | +0.01 |
| *n*-Octyl | 8.84 | 8.88 | −0.04 |

(*g*) *n*-Paraffins.

| | | | |
|---|---|---|---|
| *n*-Hexane | 9.05 | 9.08 | −0.03 |
| *n*-Octane | 8.95 | 8.96 | −0.01 |
| *n*-Decane | 8.86 | 8.88 | −0.02 |

N.B. Only one half of the compounds investigated are presented in the table.

The values of the ratio for compounds other than sulphur compounds can be calculated from the equation :

$$[P]/[R] = 8.6\ \frac{[\Sigma nvZ + \Sigma a[}{[\Sigma nv(A - Z)]}$$

where $n$ is the number of atoms of the elements (other than hydrogen) whose atomic weight, atomic number and valency are $A$, $Z$, and $v$ respectively and $a$ is a constant for each element (other than hydrogen). The values of "$a$" for the elements are :

$$C = +8\ ;\ N = -1\ ;\ O = +1\ ;\ Cl = 0\ ;\ I = 0\ ;\ Br = +2.$$

The values of the ratio for $n$-alkyl mercaptanscan be calculated from the following relation :

$$[P]/[R] = 8.5\ \frac{[\Sigma nvZ]}{[\Sigma nv(A-Z)]}.$$

The values calculated using the above relations are presented in Table I ($[P]/[R]_{der.}$). The results indicate the close agreement between the experimentally observed and the empirically calculated values.

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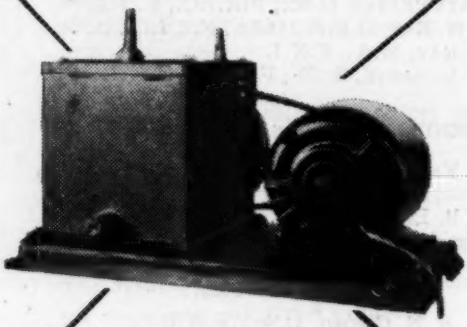

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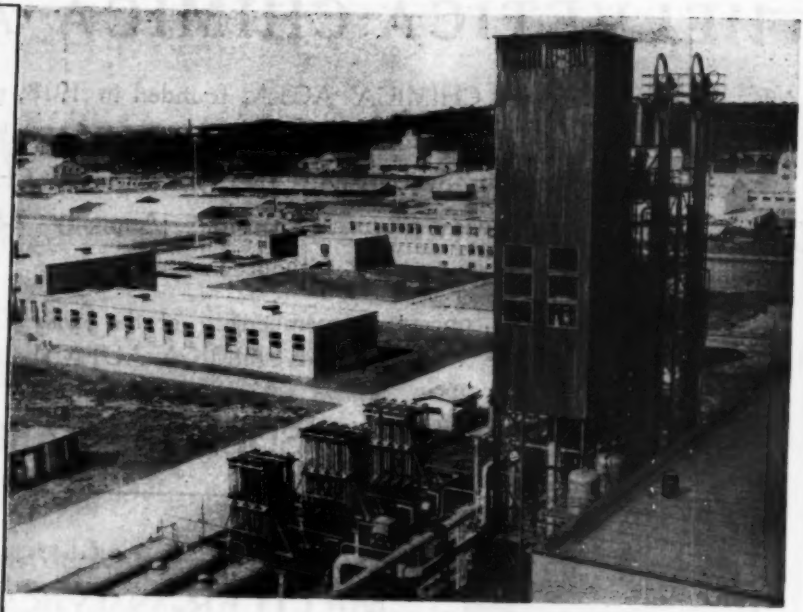

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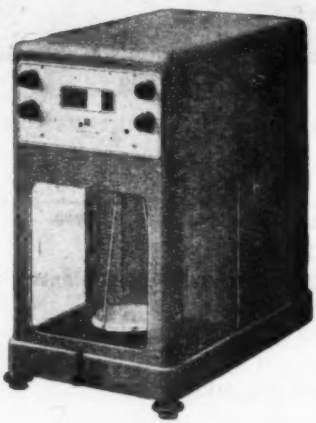

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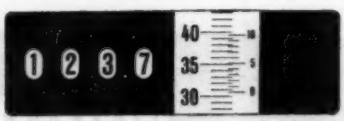


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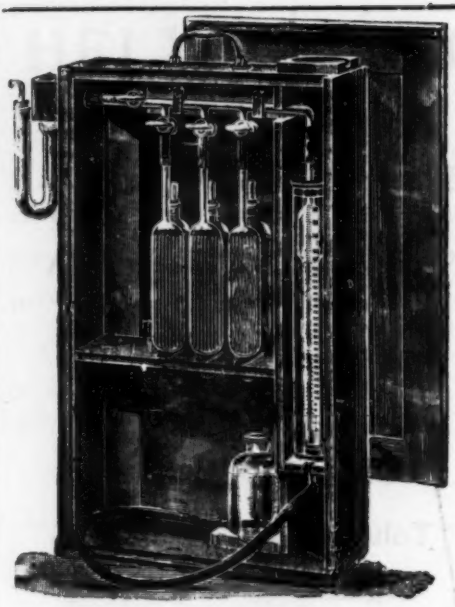

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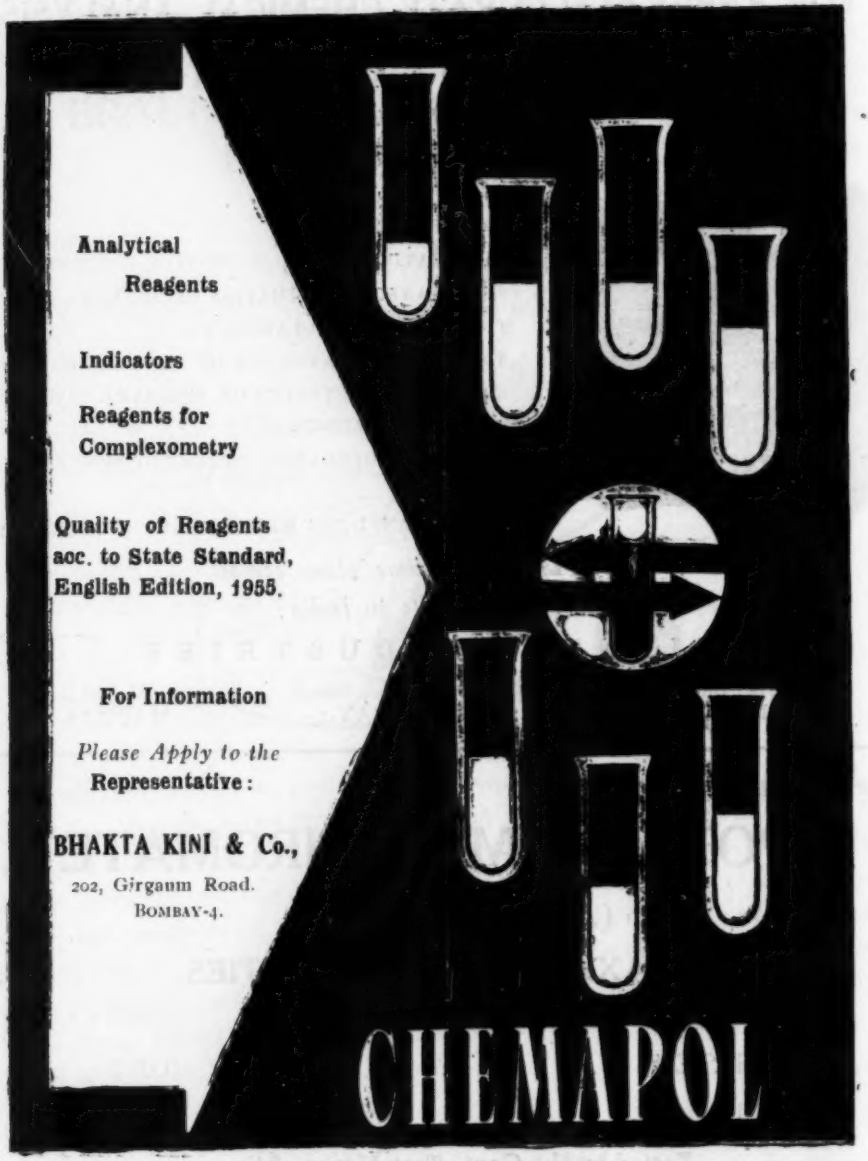


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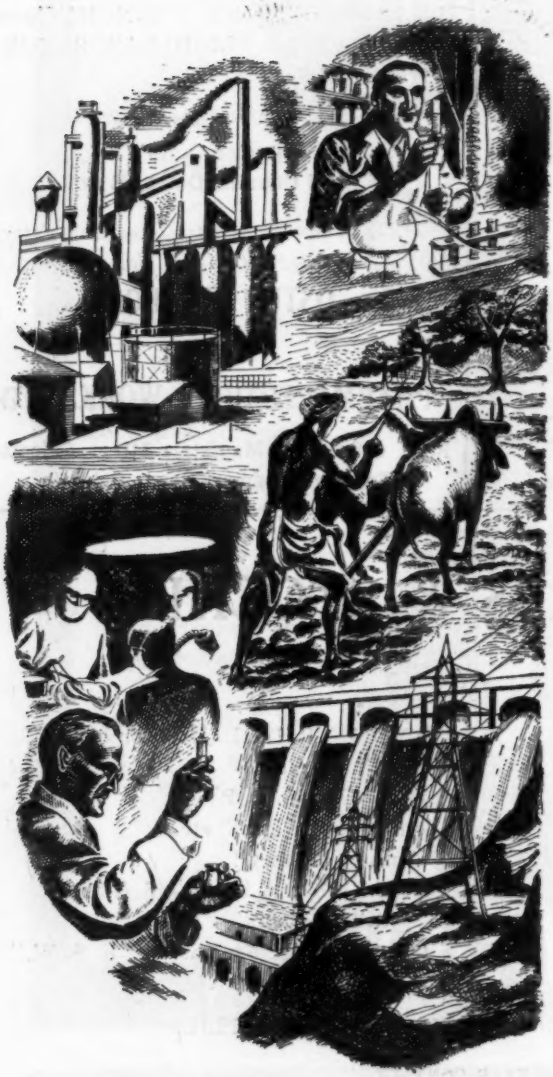

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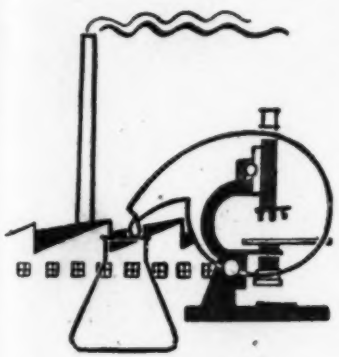

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